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CONTENTS

VOLUME TWENTY-TWO NUMBER 1
JANUARY-FEBRUARY
1956

Page

Experience with the Reddy Method for 17-hydroxycorticoids in Urine in a Clinical Chemistry Laboratory 1
by Kathleen J. Clayson, B.S.M.T. (ASCP)

Laboratory Hazards 12
by Morton Reitman, Ph.D.

Biological Hazards of Common Laboratory Procedures—III. The Centrifuge 14
by Morton Reitman, Ph.D. and G. Briggs Phillips, B.S.

Biological Hazards of Common Laboratory Procedures—IV. The Inoculating Loop 16
by G. Briggs Phillips, B.S. and Morton Reitman, Ph.D.

Useful Parasitological Methods for the Clinical and Hospital Laboratories 18
by Helen Pihl, M.S., M.T. (ASCP)

Experience with Hycel Hormone Chemistry System for Protein Bound Iodine Determinations 25
by Ida F. Levinson, M.S., M.T. (ASCP)

A Simple Method for the Quantitative Determination of Sugar in Urine 29
by Alfred S. Giordano, M.S., J. Lon Pope, M. Sc., and Bernadine Hagan, M. Sc. M.T. (ASCP)

Differential Diagnosis of Anemias Which Present Peripheral Leukoerythroblastotic Reactions 34
by R. Dorothy Sundberg, Ph.D. M.D.

Human Rabies—Diagnosis of Obscure Cases 48
by George H. Hauser, M.D. and Lucille Godelfer, M.T. (ASCP)

The Gavel 24

Bibliography of Medical Reviews 28

Helpful Hints 33

Editorial 54

The Parable of Anteen and the Wise Man 55
by Amos Cahan, M.D.

Abstracts 57

Convention Announcements 58

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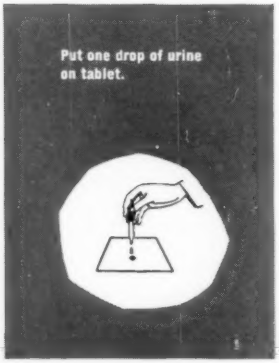

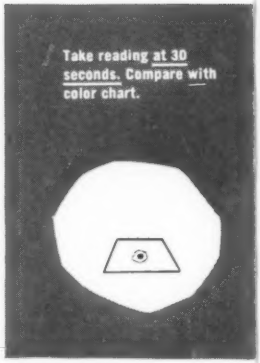

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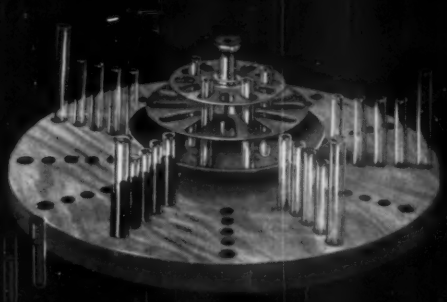

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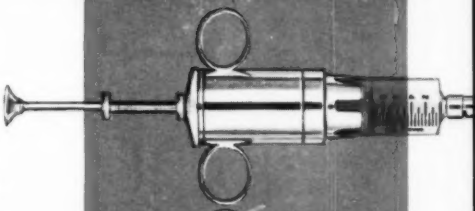

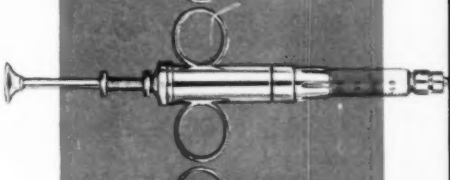

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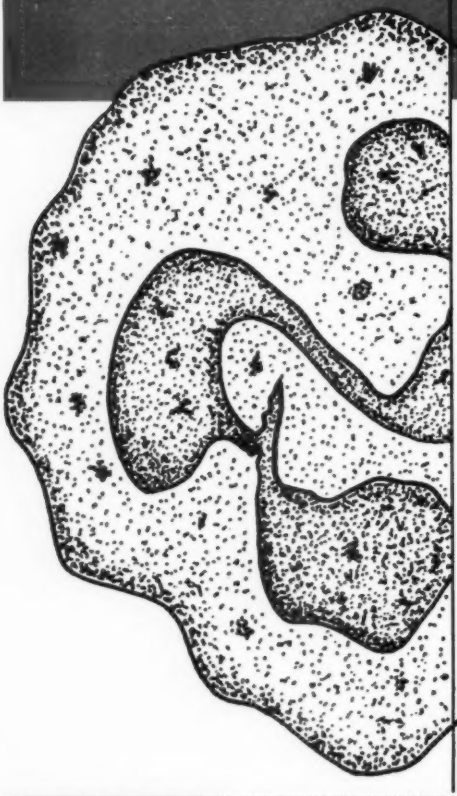

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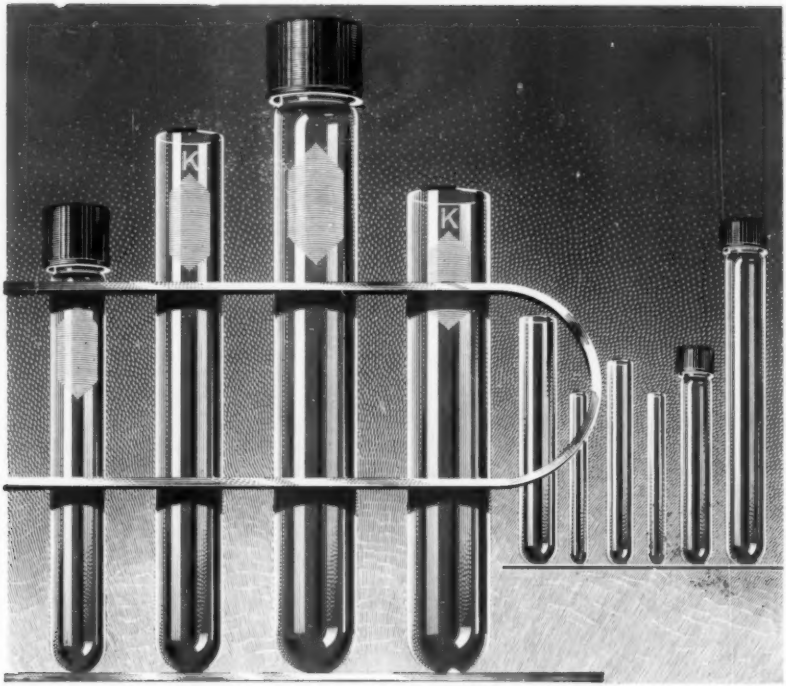

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[1] Nelson, R. A., Jr.: Science, 118: 733, 1953.
[2] Portnoy, J., and Magnuson, H. J.: J. Immunol., 75: 348, 1955.

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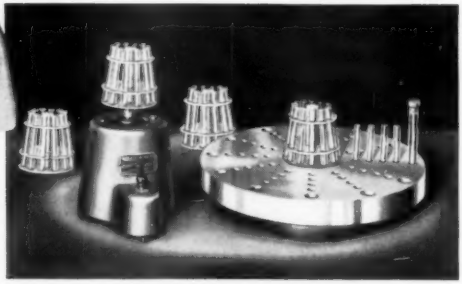

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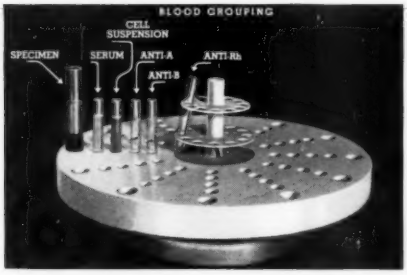

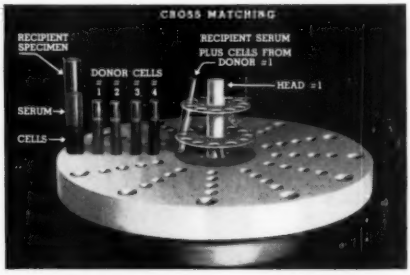

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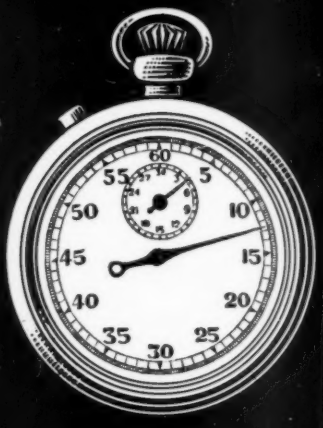

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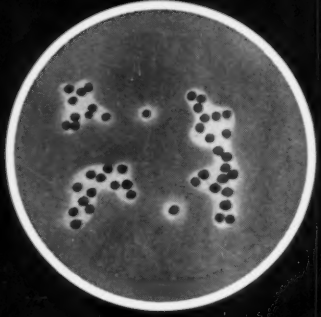

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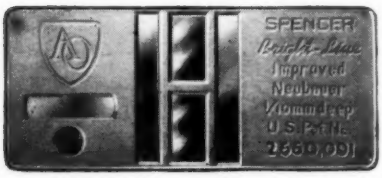

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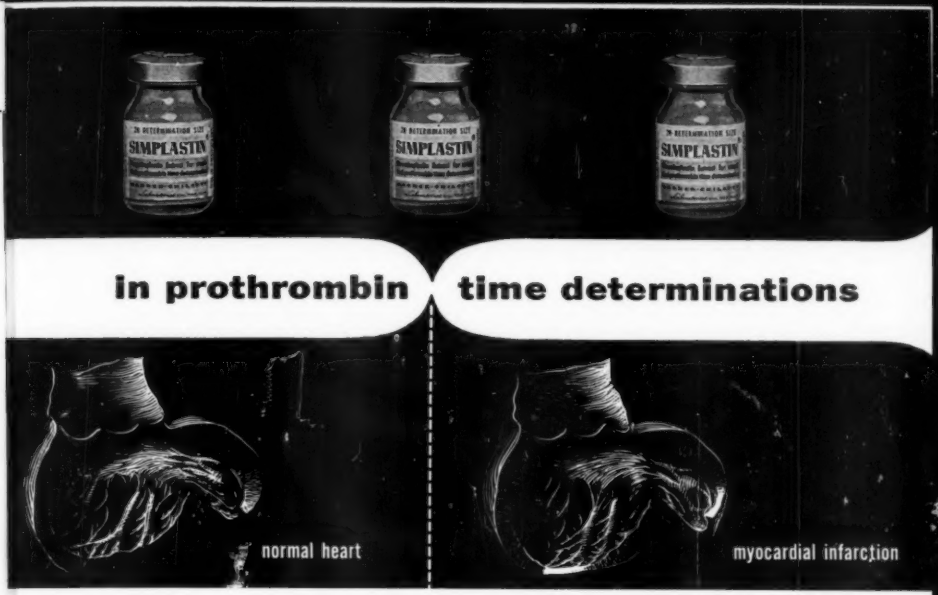

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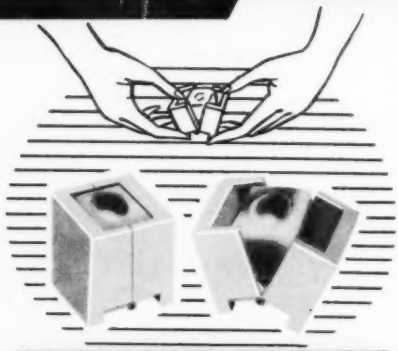

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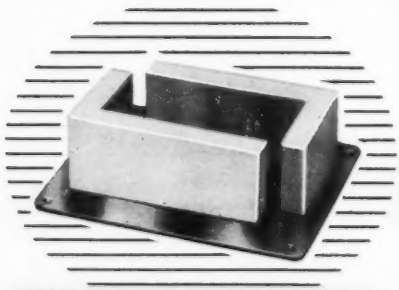

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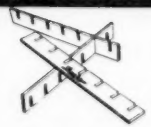

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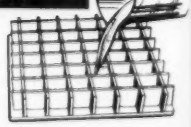

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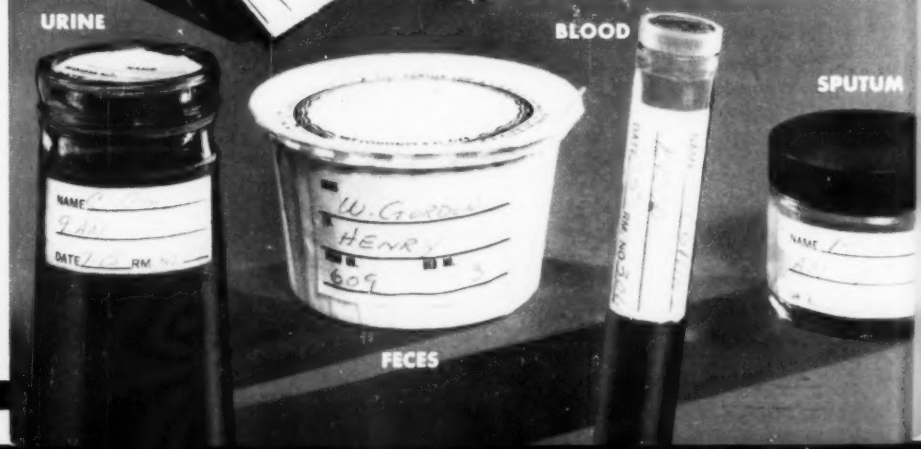

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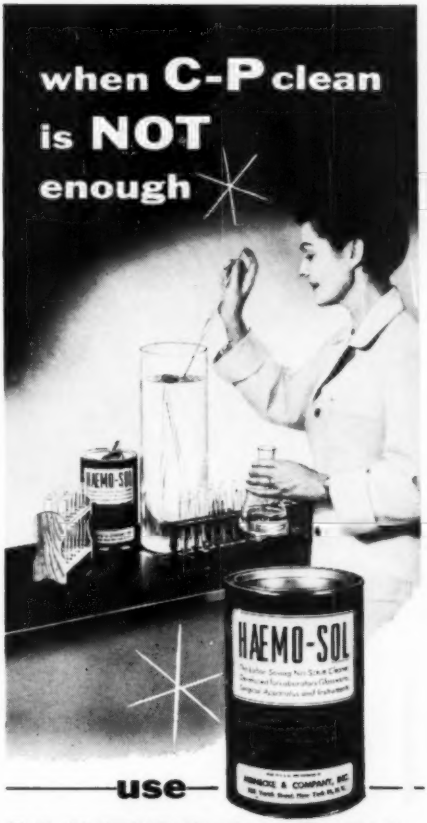

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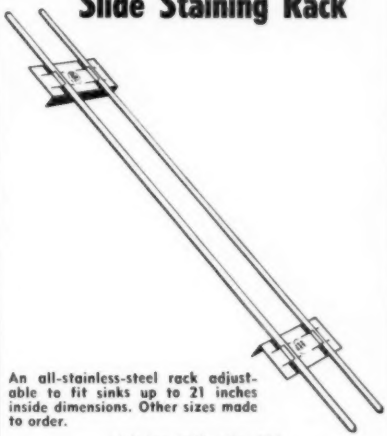

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EXPERIENCE WITH THE REDDY METHOD FOR 17-HYDROXYCORTICOIDS IN URINE IN A CLINICAL CHEMISTRY LABORATORY*

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Since Reddy, Jenkins, and Thorn[1] published their method for the determination of 17-hydroxycorticoids in urine in 1952, many groups have modified the procedure in various ways.

The principle of the method involves extraction by n-butanol of both free and conjugated 17-hydroxycorticoids from urine at pH 1.0. The Porter-Silber[2] color reaction, in which a yellow color appears in the presence of 17, 21 dihydroxy-20-ketosteroids (including cortisone and hydrocortisone) and the phenylhydrazine-sulfuric acid color reagent, is applied to the extract.

One of the major difficulties with the original Reddy et al method was the high blanks which sometimes gave negative results. For this reason many modifications by various investigators have been made, each trying in a different manner to obviate the difficulty.

After a year's experience with the method as described by Reddy, Jenkins, and Thorn[1] in 1952 and recent modifications by Smith, Mellinger, and Patti;[3] Liddle, Island, Rinfret, and Forsham;[4] and one by Reddy,[5] we now feel we have a reasonably reproducible method giving clinically useful results.

The method is relatively simple and rapid and therefore applicable to use in a clinical laboratory. This paper is concerned with a discussion of results obtained with various modifications as compared with the original method. The method which has evolved from experiences with these modifications will be presented after a discussion of each point of modification.

* Hillkowitz Award, 1955. First Award, 1955 Scientific Products Foundation, Biochemistry Division. Read before ASMT, June, 1955, New Orleans, La.

EXPERIMENTAL

Standard curve

Cortisone has been used as the reference standard in this laboratory. When hydrocortisone is used as the standard, the results will be different, but could be converted by using the ratio of their molecular weights. Porter-Silber[2] reported that the color reaction between 17,21 dihydroxy-20-ketosteroids and phenylhydrazine-sulfuric acid conforms to the Beer-Lambert Law with concentrations of cortisone up to 25μg. Reddy et al[1] reported that the curve is linear at least up to 50μg cortisone with their modification and this has been confirmed in our laboratory.

Purification of butanol

In following the original method of Reddy, Jenkins, and Thorn,[1] Merck's n-butanol was used for the extraction of the steroids from urine. However, it was found that with some patients a negative result was obtained. That is, the sum of the urine blank (extract plus 62% sulfuric acid) and the butanol blanks gave an optical density greater than that of the urine extract with phenylhydrazine-sulfuric acid reagent. The occurrence of negative results and high optical density values in the reagent blanks when using n-butanol which has not been purified in the laboratory has been reported by Smith, Mellinger, and Patti;[3] Brown, Willardson, Samuels, and Tyler;[6] and Reddy.[5] It can be seen from Table I that it is very important that the n-butanol used for extraction be aldehyde-free by purification in the laboratory. The method for purification as given in the book, *Official and Tentative Methods of Analysis of Official Agricultural Chemists*,[7] appears to be adequate and is included in the procedure as given in this paper.

This method of purification is easier than that given by Reddy.[5] The purification given by Brown et al[6] has not been tried here, but it appears to be easy and they report results comparable to those given in Table I. Smith et al[3] mentioned purification, but did not give a method other than redistillation.

Extraction of the urine at pH 1.0 versus pH 2.0-2.5.

In another attempt to decrease the optical density of the urine blanks Liddle, Island, Rinfret, and Forsham[4] adjusted the pH of the urine to 2.0-2.5 before extraction. They found that the higher pH did not affect the quantity of 17-hydroxycorticoid extracted, but did decrease the amount of non-specific pigment extracted. A comparison of the optical densities of the blanks and the final result with the urine adjusted to pH 1.0 and 2.0-2.5 was made in this laboratory and is shown in Table II. No significant difference either in final result or optical density of the urine blank was noted.

Table I
Comparison of Merck's n-butanol and laboratory purified butanol

	Merck's	Laboratory Purified
	Optical Density	Optical Density
Butanol + 62% Sulfuric Acid	.064 ± .029* (N = 26)	.004 ± .002 (N = 33)
Butanol + Phenylhydrazine Sulfuric Acid Reagent	.132 ± .022 (N = 26)	.017 ± .003 (N = 33)
Corrected Optical Density of Butanol Blanks	.069 ± .031 (N = 26)	.012 ± .002 (N = 33)
I.U. (Normal)	1.2 mg/24 hours (Had been negative on two determinations)	3.8 mg/24 hours
H.O. (Melanuria)	2.3 mg/24 hours	5.9 mg/24 hours

* Mean with standard deviation.

Table II
Comparison of extraction at pH 1.0 and pH 2.0-2.5

EXTRACTION AT pH 1.0			EXTRACTION AT pH 2.0—2.5		
O.D. of Blank	mg/24 Hrs.	Recovery	O.D. of Blank	mg/24 Hrs.	Recovery
.153	9.0	104%	.147	9.1	103%
.145	14.8	105%	.147	14.8	91%

Comparison of two acid concentrations

Smith, Mellinger, and Patti[3] claimed that there was less nonspecific color in the blanks when a more dilute sulfuric acid was used, 1.27:1 rather than 1.63:1, and the incubation time was changed from twenty minutes to forty-two minutes. Table III shows a comparison of this modification with the original Reddy method, and no improvement in the blanks is noted. The final result is higher, but it appears to be fairly consistent and therefore, it did not seem desirable to include this in a method which had been standardized at a different time.

Table III
Comparison of two acid concentrations and incubation times

1.63:1 for 20 Minutes			1.27:1 for 42 Minutes		
O.D. of Blank	mg/24 Hrs.	Recovery	O.D. of Blank	mg/24 Hrs.	Recovery
.153	9.0	104%	.154	10.2	112%
.148	14.1	97%	.151	15.4	98%
.309	19.8	103%	.349	21.8	104%
.431	11.8	92%	.461	13.9	92%

Preservation of urine

In the original Reddy et al[1] method the urine was stored in the frozen state until the determination was made. Liddle, Island, Rinfret, and Forsham[4] reported that the use of 2 ml. to 5 ml. of a 1% solution of thymol in glacial acetic acid was a preservative for urines on which 17-hydroxycorticoids were to be

determined. They report that the urine could be stored at room temperature for at least one week with no loss. To check their preservative in this laboratory, a urine specimen was divided into two parts. The preservative was added to one part and left at room temperature. The remainder of the specimen was frozen promptly. Simultaneous determinations were done on the two at later dates. Results are tabulated in Table IV. Recoveries of cortisone in butanol were consistently lower on the non-frozen specimen and the result in mg/24 hrs. was also lower than on the frozen specimen. The difference between recoveries was statistically significant. ($P = < .001$). In this laboratory urine specimens for 17-hydroxycorticoid estimation are kept in the frozen state until they are analyzed. The urine must be mixed before the determination is done to suspend the heavy precipitate which forms during thawing because the corticoid is adsorbed on the precipitate.

Table IV
Urine preservation study

	FROZEN			WITH THYMOL IN GLACIAL ACETIC ACID	
Patient	**Date**	**mg. 24 Hrs.**	**Recovery**	**mg. 24 Hrs.**	**Recovery**
I.H.	10-21-54	13.2	102%	12.5	86%
	10-29-54	14.0	97%	13.2	86%
	11-22-54	13.1	90%	12.1	80%
N.L.	11-23-54*	6.4	95%	...	...
	12-23-54	6.5	99%	5.9	87%

Recovery: Mean for frozen sample..... 96.6 ± 4.5
Mean for sample + preservative. 85.0 ± 3.2

* Before freezing.

Order of adding reagents

Smith et al[3] use a modification in which the 1 ml. aliquots of extract are added to 4 ml. of reagent already in the tubes. The tubes are kept in an ice bath until all are ready for incubation. This modification is faster and results more reproducible than when the reagent is added to the extract.

Saturation of urine with sodium sulfate

In Reddy's[5] modification in 1954, he proposed saturating the urine with sodium sulfate to prevent difficult emulsions. The results obtained in our laboratory by saturating the urine with sodium sulfate before the first extraction as compared with shaking the combined extracts with sodium sulfate are given in Table V. Use of the sodium sulfate saturation before the first extraction as given in Reddy's modification simplified the method since emulsions are not formed and albuminuria does not interfere. Therefore this is included in the method used in this laboratory.

Table V
Comparison of saturation of urine with sodium sulfate before extraction with shaking combined extract with sodium sulfate

COLUMN I Saturation with Sodium Sulfate Before Extraction	COLUMN II Shaking Extract with Sodium Sulfate After Extraction	Percent Difference Between Columns I and II Over Column II
37.1	40.5	— 8.4
29.5	32.0	— 7.8
34.4	40.9	—15.9
24.3	28.6	—15.0
13.2	13.4	— 1.5
28.9	26.5	+ 9.1
26.0	24.1	+ 7.9
21.6	20.8	+ 3.8
46.5	43.4	+ 7.1
40.5	39.3	+ 3.1
30.6	30.3	+ 1.0
	Mean Percent....	— 1.5

Number of extractions

The 1954 Reddy modification also eliminated a second butanol extraction of the urine substituting one five minute shaking period for the two-three minute extractions in the original method. In Table VI we show the comparison between the two extraction procedures that was found here. From Table VI it appears that two extractions are better than one. Therefore in the procedure to be given later in this paper it was decided to retain the double extraction procedure of the original Reddy et al[1] method.

Table VI
Comparison of one extraction versus two extractions

Two Extractions with 5 ml. Butanol (Shaking 3 Minutes each Time)	One Extraction with 10 ml. Butanol (Shaking Time 5 Minutes)
40.5	37.1
32.0	29.5
42.6	49.8
41.8	40.3
14.2	14.3
14.5	17.2
16.8	14.4

Extraction of different size aliquots of urine

Our experience with this method has shown that extraction of varying amounts of urine from the same specimen give the same result with some patients and not with others. Mason[8] has reported that small aliquots of urine may give higher values than larger aliquots in some specimens. He was using the formaldehydogenic method, but reported that others found similar results when reducing substances were measured. To our knowledge this has not been reported as happening with the Reddy method. For an idea of the kind of variation that can occur

when using different size aliquots see Table VII. It is not known why this happens and there seems to be no way of predicting whether different aliquots on a given patient will agree. Unfortunately recoveries of added cortisone will not indicate the trouble because they are satisfactory even on those specimens in which values on small and large aliquots do not agree. For this reason two different size aliquots are used for each patient. If the two aliquots give comparable results, that answer can be reported. If the two results are different, smaller aliquots are used until two agree.

Table VII

Variation of value of 17-hydroxycorticoids with the volume of urine used for extraction

Patient	Size of Aliquot (ml.)	17-Hydroxycorticoid (mg. 24 Hrs.)
A. Those with Acceptable Agreement Between Aliquots.		
D.K.	5	7.9
	10	8.3
	20	7.6
F.H.	10	4.0
	20	4.0
V.G.	5	2.9
	10	2.4
M.G.	10	1.9
	20	2.5
E.P.	10	10.0
	20	11.6
H.H.	1	24.4
	2	26.4
	3	26.0
	5	24.5
	10	22.9
D.G.	1	43.8
	2	42.2
	3	42.4
	5	42.8
	10	41.7
K.M.	5	4.9
	10	4.4
Those Urines Which Give Higher Values with Small Aliquots.		
C.K.	3	4.7
	4	4.3
	5	3.2
	10	3.1
T.M.	5	3.3
	10	2.6
	20	2.3
B.F.	1	44.1
	2	44.5
	3	41.0
	5	31.0
	10	21.0
	20	14.3

The method used in this laboratory as the result of this experimental work is now presented.

METHOD

Reagents

1. N-butanol (reagent grade). Purification. (aldehyde-free alcohol).[7] Redistill alcohol over sodium hydroxide or potassium hydroxide, (add about ten pellets per liter of butanol), add 2-3 gm. per liter of metaphenylenediamine hydrochloride, digest at ordinary temperatures several days (or under reflux condenser on steam bath several hours), and distill slowly rejecting the first 100 ml. and last 200 ml. of distillate.
2. Fifty percent sulfuric acid (reagent grade).
3. Sixty-two percent sulfuric acid (1.63:1). (reagent grade). Add 620 ml. concentrated sulfuric acid to 380 ml. water in a pyrex flask. Cool. Transfer to a 1 liter volumetric flask and dilute to volume with distilled water.
4. Phenylhydrazine hydrochloride. Dissolve phenylhydrazine hydrochloride in a minimal amount of absolute alcohol by heating. Allow to cool at room temperature. Refrigerate for at least one hour. Filter through a sintered glass funnel. Transfer crystals, which should be peach-colored, to a clean container and place in desiccator.
5. Phenylhydrazine-sulfuric acid reagent. Dissolve 65 mg. of phenylhydrazine hydrochloride in 100 ml. of 62% sulfuric acid.
6. Anhydrous sodium sulfate.
7. Anhydrous sodium carbonate.
8. Cortisone standards.* (11-dehydro-17 hydroxycorticosterone)

   Stock (500μg/ml.)

   50 mg. cortisone diluted to 100 ml. with purified n-butanol. Requires several hours for solution.

   Working

   1 ml. stock diluted to 50 ml. in n-butanol (10μg/ml.)

   5 ml. stock diluted to 100 ml. in n-butanol (25μg/ml.)

   5 ml. stock diluted to 50 ml. in n-butanol (50μg/ml.)

The standards are stable indefinitely at refrigerator temperatures.

Procedure

Estimation of total 17-hydroxycorticoids.

Use a 24 hr. urine specimen that has been collected on ice without a preservative. The specimen can be stored in a deep freeze indefinitely.

A. Extraction and purification:

1. Two different size aliquots of urine, i.e. 5 ml. and 10 ml., are used. Place in 40 ml. round bottom centrifuge tubes fitted with standard taper ground glass stoppers.

   Recovery determinations can be carried out by adding a known amount of cortisone in water to the aliquot of urine

* Cortisone provided by the Glidden Company and the Upjohn Company.

or by adding a known amount of cortisone in butanol with the butanol at the first extraction step.

2. Adjust the total volume to 10 ml. with distilled water.
3. Adjust the pH to 1.0 with indicator paper by the dropwise addition of fifty percent sulfuric acid. The pH can be checked with a pH meter.
4. Saturate the urine with sodium sulfate by adding 2-3 gms. before the first extraction.
5. Add 5 ml. purified n-butanol and shake for three minutes. Centrifuge if necessary. Transfer the butanol layer to a clean 40 ml. centrifuge tube.
6. Repeat the extraction with another 5 ml. of butanol.
7. Shake the combined butanol extracts with approximately 1 gm. sodium carbonate. Centrifuge and decant the butanol extract to a clean tube.
8. Colorimetry should be performed immediately.

B. Color development: Porter-Silber reaction.

20 x 175 mm. tubes are used for the color development.

In an ice bath:

Butanol blank (A) 1 ml. butanol 4 ml. sulfuric acid (1:63:1)	Standard blank (A) 1 ml. standard 4 ml. sulfuric acid (1:63:1)	Unknown blank (A) 1 ml. extract 4 ml. sulfuric acid (1:63:1)
Reagent blank (B) 1 ml. butanol 4 ml. color reagent	Standard (B) 1 ml. standard 4 ml. color reagent	Unknown (B) (in triplicate) 1 ml. extract 4 ml. color reagent

Two or three standard concentrations are included with each determination.

1. Mix immediately after the addition of the butanol, standard, or extract to the reagent.
2. Keep in an ice bath until all have been added to the reagent, then transfer to a constant temperature water bath maintained at 60° C. ± 1° C. for twenty minutes.
3. Transfer to a cold water bath for five minutes.
4. Read in the Beckman Model DU spectrophotometer at a wave length of 410 millimicrons set at 0 optical density with a sulfuric acid (1.63:1) blank.

C. Calculations:

Reagent blank B (optical density)—Butanol blank A (optical density) = a

Standard B (optical density) — Standard A (optical density) = b

Unknown B (optical density) — Unknown A (optical density) = c

b-a = corrected optical density for the standards. Plot the

corresponding galvanometer reading on semi-logarithmic graph paper against cortisone in μg.
c-a = corrected optical density for the unknown.

$$\text{Reading from graph} \times \frac{10 \text{ (total extract)}}{1 \text{ (extract used)}} \times \frac{\text{total urine volume}}{\text{aliquot used}} \times \frac{\mu\text{g cortisone}}{1000} = \text{mg/24 hrs.}$$

RESULTS

Precision of the method

Extractions on all urines have been done on duplicate aliquots. The precision of the method based on the average difference between 83 sets of duplicates indicates a standard deviation of $\pm$ 0.43 μg. The mean value for the 83 urine specimens is 9.63 μg with a range of 0.40 μg to 69.60 μg. The percentage error based on the mean of the group is 5%. Naturally the percentage error will be greater on lower values and smaller on high ones.

At the colorimetric stage all blanks (urine extracts plus 1.63:1 sulfuric acid) were done in duplicate. The average difference in duplicates is 0.002 optical density units based on 122 sets of two indicating a standard deviation of $\pm$ .0018 for any one reading. One extract-acid blank gives adequate precision and the duplicate was eliminated.

The extract plus phenylhydrazine-sulfuric acid reagent has been set up in triplicate. Based on 122 sets of three the average range in optical density is 0.006 giving a standard deviation of $\pm$ .0053.

Recoveries

Two types of recoveries have been done.

1. Cortisone (100 μg) in n-butanol was added with the n-butanol to the urine before the first extraction. Therefore with a total extract volume of 10 ml. the expected recovery in 1 ml. of extract is 10 μg. The mean recovery is 96.9% (based on 92 determinations) with a standard deviation of $\pm$ 8.4%.
2. Cortisone (110.7 μg) in water was added to the urine after adjusting pH to 1.0 and before the first extraction. With a total of 10 ml. of extract the expected recovery from 1 ml. of extract is 11.07 μg. The mean recovery is 101.6% with a standard deviation of $\pm$ 6.7% (N = 21).

Normals

A series of normals has not yet been done with the method as described in this paper. It is felt that the range will probably be similar to that of Reddy[5] which is 1.1 to 10.7 mg/24 hrs. Determinations of 17-hydroxycorticoids on a group of normals will be done so that a range can be established for this laboratory.

Table VIII
Agreement between 17-hydroxycorticoid result and clinical diagnosis

Patient	Diagnosis	17-Hydroxycorticoid (mg/24 Hrs.)
P.V.	Hypopituitary Function	1.9
	After ACTH	13.6
I.H.	Adrenal Tumor	14.8
B.F.	Cortisone Therapy	44.4
H.H.	Cortisone Therapy	25.4
D.G.	Cortisone Therapy	42.5

Clinical Experience

That this method is clinically useful can be seen in Table VIII. A patient with hypopituitary function shows a borderline low result and patients with adrenal tumors and hyperplasia show elevated results. Patients on cortisone therapy have increased urinary excretion.

PRECAUTIONS

Just recently Marks and Leftin[9] have reported that potassium iodide, iodine, chloral hydrate, and paraldehyde react with phenylhydrazine-sulfuric acid to give a colored product with maximum absorption at 410 mμ which is the same as that of the 17, 21 dihydroxy-20-ketosteroids. Wolfson[10] has reported that quinidine and propylene glycol are also measured at that wavelength. It is important that the clinician be aware of these interfering substances so that a 17-hydroxycorticoid determination is not done when the patient is receiving any of them.

Either a Beckman or Coleman spectrophotometer has been used by everyone who has published work involving the Porter-Silber reaction for the measurement of 17-hydroxycorticoids. The Evelyn colorimeter did not prove satisfactory when tried here and it probably indicates that no filter photometer is adequate for this method.

OTHER METHODS

There are other modifications of the Reddy method which have not been discussed here. Brown[11] and Liddle et al[4] take an aliquot of the butanol extract to dryness and dissolve the residue in methanol for the Porter-Silber color reaction. They report lowered urine blanks. Schneider[12] has changed the washing procedure and conditions for color development so that he claims higher results and lower blanks are obtained. These modifications have not been tried because we are satisfied with the method as presented here.

SUMMARY

Experimental comparisons between the original Reddy, Jenkins,

and Thorn method for determination of 17-hydroxycorticoids in urine and various modifications of that method are made. The method which has evolved from experiences with these modifications is presented after a discussion of each point of modification.

Data on the precision of the method and recoveries of added cortisone are included.

ACKNOWLEDGMENT

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LABORATORY HAZARDS**

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Infections acquired in the laboratory have taken their toll of scientists.[1, 2] Among the organisms which have been implicated as the causative agent in fatal laboratory infections are: *Brucella melitensis*[3,4,5] *Shigella dysenteriae*,[6] *Bacterium tularensis*,[7] *Salmonella typhosa*,[1] poliomyelitis virus,[8] yellow fever virus[9] and *Rickettsia rickettsii*.[10]

In a survey of 1342 laboratory-acquired infections in the United States there was a case fatality rate of 3 per cent. Recognizable accidents in the laboratory accounted for only 16 per cent of the total infections.[11] Our laboratory has been concerned with the possible causes of the other 84 per cent and for the past eight years we have been investigating the infectious hazards of laboratory techniques. The results of these investigations have been released in the form of publications,[12, 13, 14, 15, 16] and a series of films and film strips* has been produced in cooperation with the Communicable Disease Center at Chamblee, Ga.

All manipulations which are done in a routine day in the microbiological laboratory result in the production of aerosols which contaminate the environment. Whether or not the laboratory technician becomes infected depends upon the *degree* of contamination, *virulence* of the organism, and the *resistance* of the individual. We can reduce contamination by improving techniques and by designing leakproof apparatus, but we can best eliminate the *source* of infection by inclosing the working area in a *ventilated cabinet*.[13] For example, a simple technique such as allowing culture fluid to slowly drain down the side of a test tube instead of dropping directly into the center of the tube will practically eliminate this source of aerosol. Of course, the last drop in the pipette should never be blown out. A leakproof blendor[17, 18] has been designed by our laboratory and is now in use. Although the blendor is not yet available to the public, we have hopes that it will be produced commercially in the near future. There are now many bacteriological safety cabinets on the market. These cabinets designed by our laboratory are modified radiological cabinets. The technician is protected against infectious aerosols by the insertion of a pane of glass between himself and the working area; a blower continually sweeps contaminated air away from the technician. All air leaving the cabinet is filtered through a specially designed spun glass filter. Facilities such as water, air, vacuum, and gas are

* Infectious Hazards of Bacteriological Techniques—sound film.
Infectious Hazards of Bacteriological Techniques—film strip. Parts I-V.

** Read before ASMT, June, 1955, New Orleans, La.

available in the cabinet.

When it is at all possible personnel should be immunized against the organisms which are being handled. Immunization should be kept at a high level by frequent booster shots. At the first signs of illness an occupational disease should be suspected until ruled out by diagnostic evidence.

Careful planning of procedures together with proper use of available safety equipment will prevent infections and allow studies of highly infectious disease agents which hitherto have been taboo due to the hazards involved.

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BIOLOGICAL HAZARDS OF COMMON LABORATORY PROCEDURES*

III. The Centrifuge

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This article, the third in a series, describes certain of the potential infectious hazards of the centrifuge. Experiments are included in which tubes in the revolving centrifuge were caused to break or shatter. In contrast to the inapparent hazards of some procedures with the pipette (Reitman and Phillips, 1955) and hypodermic syringe and needle (Hanel and Alg, 1955), the high centrifugal forces and open construction of most centrifuges indicate the danger accompanying accidents in it. These hazards have been evaluated as well as the hazards of other centrifugation procedures.

METHODS

The methods used were the same as those previously described (Reitman and Phillips, 1955). *Serratia indica* was the test organism. Air samples at an air flow of 1 cu. ft. per minute were taken with sieve type air samplers positioned usually 6 to 8 inches above the tube or centrifuge. Control air sampling revealed none of the test organisms in the air before each test. An International centrifuge*, model SV 2, with a slotted, swing type head suitable for 50-ml tubes was used for all studies.

RESULTS

Filling Centrifuge Tubes—A direct relationship was found between the amount of culture placed in a centrifuge tube and the extent of aerosol produced. When 10- and 30-ml of *S. indica* culture were carefully dispensed down the sides of 50-ml centrifuge tubes with volumetric pipettes, an average of 0.6 and 1.2 clumps of organisms per tube, respectively, were recovered from the air above the tubes.

Removal of Cotton Plugs and Rubber Caps from Tubes After Centrifuging—The removal of cotton plugs from centrifuged tubes, even if the cotton is dry, releases an aerosol. The amount of the aerosol depends on the technique of the individual. When 100 plugs were removed in 10 separate tests by one operator, an average of 2.3 clumps of organisms per plug was recovered from the air.

The use of rubber caps (Sani-Tab caps**) on the centrifuge tubes greatly reduced the aerosol. When 100 caps were removed in 10 tests by one operator, no organisms were recovered. When 80 caps were removed in 10 tests by a second operator an average of only 0.2 clumps of organisms was recovered per cap.

* International Equipment Co., Boston, Mass.
** Macalaster Bicknell Co., Inc., New Haven, Conn.

Removal of Supernatant With a Siphon—Air was sampled during the removal of supernatant with a micropipette siphon from 10 tubes containing 30 ml of centrifuged culture. An average of 0.3 clumps of organisms was recovered per tube.

Resuspending Centrifuged Cells—Adding 30 ml of resuspending fluid to the packed cells and resuspending the cells by mixing with a pipette created an aerosol. An average of 4.5 clumps of organisms was recovered per operation during 10 separate tests.

Breakage of Centrifuge Tubes—The greatest hazard associated with centrifuging infectious materials is that of breakage of tubes. Two types of breakage may occur: (1) tubes may crack or break in the carrier cup but the fluid remains in the carrier cup by centrifugal force or (2) the tube may shatter and release the culture into the centrifuge chamber which then disseminates the culture throughout the room. Few aerosolized bacteria are released into the laboratory air by the former type of breakage but a large number are released by the latter.

Air sampling during type 1 breakage of a 50-ml centrifuge tube containing 30 ml of *S. indica* culture, repeated 10 times, resulted in an average recovery from the room air of 4 clumps of organisms per breakage. Similar breakage of 10 tubes by the second method gave an average aerosol recovery of 1,183 per tube. Type 1 breakage is apt to be an insidious danger because the breakage may not be apparent to the operator until he attempts to remove the tube from the carrier.

Table 1
Hazards of Centrifuging Procedures

Technique	Cu. Ft. of Air Sampled*	Average Number of Clumps of Organisms Recovered From Air During Technique
1. Filling centrifuge tubes, 10 ml culture	1	0.6
2. Filling centrifuge tubes, 30 ml culture	1	1.2
3. Removal of cotton plugs after centrifuging	2	2.3
4. Removal of rubber caps after centrifuging	2	0.2
5. Removal of supernatant from 30 ml of centrifuged culture	1	0.3
6. Resuspending cells from 30 ml of culture	1	4.5
7. Breakage of tubes, (1) cracked tube	10	4.0
8. Breakage of tubes, (2) shattered tube	10	1,183.0

* Air was sampled at the rate of 1 cubic foot per minute.

PROTECTIVE MEASURES

The best protection for centrifuging operations is afforded by the bacteriological safety cabinet (Wedum 1953) for filling and emptying tubes, accompanied by the use of commercially available safety trunnion cups, caps, inserts, and special angle heads.**

** International Equipment Co., Boston, Mass.

SUMMARY

As summarized in Table I, the filling of centrifuge tubes with cultures or suspensions of bacteria, the removal of cotton plugs after centrifuging, and the resuspending of bacterial cells resulted in formation of minor bacterial aerosols. Shattering of a tube of culture in the centrifuge caused a voluminous bacterial aerosol to be liberated into the laboratory.

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BIOLOGICAL HAZARDS OF COMMON LABORATORY PROCEDURES

IV. The Inoculating Loop

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The bacteriologist uses the inoculating loop for a variety of manipulations involving the transfer of cultures and the inoculation of media. Lengths of platinum or nichrome wire are inserted into a suitable holder and a small loop, usually about 3-4 mm in diameter, is formed in the distal end of the wire. The frequency and variety of use of the loop in the infectious disease laboratory justifies an investigation of the hazards involved.

For these tests the procedures commonly performed with the inoculating loop were separated into single operations and each operation was investigated separately. The methods used were the same as previously reported (Reitman and Phillips 1955). *Serratia indica* was the test organism. Appropriate control air sampling revealed none of the test organisms in the air before each test.

RESULTS

A summary of the results is shown in Table 1.

Flaming of a loop was tested in 4 ways as shown in the table. A Fisher burner was used in all tests. Despite all the sputtering and splattering accompanying the flaming of a loop, this is a relatively safe procedure. Only rarely were aerosolized bacteria recovered by air sampling or by sampling the desk top around the base of the burner. However, insertion of a **hot** loop into 100 ml of broth culture in a 250-ml Erlenmeyer flask produced a bacterial aerosol. Sampling the air about the mouth of the flask by means of a sieve air sampler enabled the recovery of

Table I
Hazards involved in use of the inoculating loop

Technique	Average Number of Clumps of Organisms Recovered From the Air During Technique
1. **Flaming an Inoculating Loop:**	
(a) Loopful of milk culture in a flame*	0
(b) Blood mixed with bacterial culture*	0.01
2. **Insertion of an Inoculating Loop into Broth Culture:**	
(a) Hot loop inserted into broth culture in test tube	0.1
(b) Hot loop inserted into broth culture in 250 ml flask	8.7
(c) Cold loop inserted into broth culture in test tube	0.1
(d) Cold loop inserted into broth culture in 250 ml flask	0.8
3. **Bursting of the Film in an Inoculating Loop**	0.2
4. **Streaking Agar Plates:**	
(a) Inoculum from an agar colony	0.1
(b) Inoculum from broth	0.2
5. **Shaking Loopful of Culture in a Test Tube**	0.5

* 250 needle flamings, 50 in each of 5 different positions of the needle in the flame.

an average of 8.7 clumps of bacteria per operation. Allowing the loop to cool before insertion into the broth culture reduced the recoverable aerosol to an average value of 0.8 colonies (Anderson *et al*, 1952).

The technique of shaking a loop as it is withdrawn from a culture tube to remove excess inocula yielded an average of 0.5 colonies per operation.

When transferring liquid cultures, the film of liquid on the loop often bursts accidentally. When such films were intentionally broken by touching with a needle, each operation resulted in the recovery of 0.2 *S. indica* colonies from the air.

Detectable aerosols were also recovered when agar plates were streaked with inocula. Streaking smooth agar plates rarely looses bacteria into the air but when the agar surface is rough or if careless technic is used, it is obvious that the loop may be caused to vibrate thus creating an aerosol. The use of a bent glass rod to spread inocula over the surface of smooth agar plates will greatly reduce aerosolization.

SUMMARY

Of the inoculating loop techniques investigated, insertion of a hot loop into a flask of broth culture appeared to create the greatest hazard. The aerosol is reduced if a cold loop is used.

It is significant that some aerosolized bacteria were recovered during almost all of the techniques tried.

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USEFUL PARASITOLOGICAL METHODS FOR THE CLINICAL AND HOSPITAL LABORATORIES

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The effective detection of intestinal parasites in feces depends upon several factors including: 1) the freshness of the specimen; 2) the promptness with which it is examined after being received in the laboratory; 3) the amount of time that can be used in the search; 4) the infection suspected; and 5) the procedures employed.

Since there is no single examining technic which is likely to reveal all parasites in a search, several may have to be employed. This is especially true of light infections or those in between periods of activity in any infection when few cysts or ova can be found.

Following are the methods which have been successfully employed in a modern hospital laboratory and are presented here in the hope that other laboratories will find them useful.

COLLECTION OF SPECIMENS

A. **Hospital patients:**

1. Amoeba searches:

a. A purge may be given (unless contraindicated) following the collection of a normally passed specimen. Theoretically, this enables the technologist to examine formed, semi-formed to semi-fluid, and more fluid specimens from each patient.

Motile trophozoites are usually found in mushy or semi-fluid stools while the formed or semi-formed stools yield the cysts of these organisms.

As a purge Fleet's phosphosoda is recommended. One ounce, with copious amounts of water, is given before breakfast, followed by breakfast and coffee. Ambulation is encouraged if the patient is able. Three or four stool specimens are collected at intervals of at least 20 minutes. The patient is instructed not to urinate into the bedpan from which the specimens are collected.

b. In the event a cathartic is contraindicated and the stools are solid, specimens are sent to the laboratory daily. One-half pint of lukewarm normal saline may be given as an enema to encourage movement.

c. If, on the other hand, the patient is having spontaneous diarrhea, normally passed specimens are collected as produced until the desired number have been examined.

Collection of the specimens and subsequent delivery to the laboratory are the same in all cases. All, or a portion, of each

specimen, is sent to the laboratory **immediately**. If a portion of the stool is selected, it should be representative of the stool with respect to color, consistency, mucus, gross blood, etc. The most satisfactory type of container is the half pint paraffined ice cream carton with overlapping cover.

It is an excellent idea to learn the extent of the patient's travels. This knowledge helps to determine subsequent examining procedures.

2. Worm searches: From patients suspected of having helminths, other than pinworms, normally passed specimens are collected. Purges are usually not given at the time of the search. Anal swabs are used in the hunt for pinworms or their ova.

B. **Outpatients**: Outpatients may be of two types; 1) the patient who comes to the laboratory for a purge and subsequent delivery of three to four specimens on the same day, or 2) the patient who daily brings a fresh normally passed specimen from home to the laboratory. The latter are asked to discharge their specimens directly into suitable containers (screw top jars or cartons) so as not to let them come in contact with water or urine, to wrap them well in newspaper for insulation and to deliver them to the laboratory as quickly as possible.

In the event that no parasites are found in the first series of specimens examined and the physician is still of the opinion that the patient is infected, he may wish to have the work repeated one or several times.

MEDICATION INTERFERING WITH PARASITOLOGICAL EXAMINATIONS

Patients must NOT have had any of the following for at least ten days prior to the collection of stools:

A. Barium, Bismuth (incl. Pepto Bismol, Kaopectate, etc.)

B. Amebacidal agents such as Emetine, Aralen Phosphate, Vioform, Carbarsone, Milibis, Diodoquin, etc.

C. Antibiotics, including Penicillin, Streptomycin, Aureomycin, Bacitracin, Terramycin, etc.

D. Laxatives: Castor oil, mineral oil, psyllium seed including Metamucil and Mucilose.

E. Glycerine suppositories.

PROCESSING OF SPECIMENS IN THE LABORATORY

After delivery to the laboratory specimens are kept at room temperature until they have been examined. Incubator storage, if used for fluid specimens, should be for a short time only (no more than 30 minutes) since increased bacterial multiplication and gas formation shortly destroy the protozoan organism.

A. **Macroscopic examination (each specimen):** The color, consistency, gross blood, mucus, pus and undigested food are noted and a careful search for adult organisms such as pinworms, roundworms, hookworms and the proglottids of tapeworms is made.

To demonstrate small helminths, it is sometimes necessary to mix a portion of the feces, the size of a walnut, in tap water and pour the mixture slowly through a sieve of about 30-50 mesh. The sediment is washed with a small stream of lukewarm water and examined.

B. **Microscopic examination (each specimen):** Temporary mounts are made in saline. If many specimens are delivered to the laboratory in a short space of time the soft or mushy ones are searched first. However, there should be as little delay as possible in examining formed stools since trophozoites are sometimes found on the outer surfaces.

Samples for microscopic examination are taken from several portions of the stool. The more heterogeneous the specimen, the more samples are taken. Small flecks of blood, mucus and tissue detritus are carefully examined. Taken from the outer surface of a fecal mass, they may be the only portions of the sample to exhibit evidence of parasitic infection. Material from liquid feces is withdrawn by means of a capillary pipette from the bottom of the container.

Formed specimens can be stored in the refrigerator for several days or longer for future examination. They must not be allowed to dry out.

Trophozoites in saline are not always immediately active. As a stimulus to motility, 1) the slide of a fresh preparation in saline can be warmed by placing it upon the stage of the microscope which in turn is heated by means of a towel covering both the microscope and the lamp; 2) an anaerobic condition may be created by ringing the coverslip with a half and half mixture of melted paraffin and petroleum jelly.

Ringing of the coverslip also prevents drying of the slide, and, by anchoring the coverslip the use of the oil immersion objective is facilitated.

Wet staining procedures of fresh material enhance the study of amoebae. For trophozoites Velat's stain[4] (a buffered aqueous solution of the precipitate formed from the interaction of hematoxylin and crystal violet) is unusually good. Nuclei are delicately colored as well as sharply defined.

An iodine solution is valuable for detecting cysts but has the disadvantages of often destroying trophozoites completely and of sometimes coagulating the proteins in the stool. The latter

action undoubtedly hides organisms by drawing them into the fecal clumps.

Smears of feces in tap or distilled water in place of saline may be used as an aid in identifying Dientamoeba fragilis. Examine immediately. Blastocystis hominis is destroyed. Other trophozoites disintegrate while Dientamoeba fragilis swells, then ruptures but immediately regains a spherical shape enclosing small particles in brownian movement. This action is known as Hakansson's phenomenon.

Other than the sought for protozoan trophozoites and cysts, helminth ova, larvae and adults, the microscopic examination of feces reveals other significant objects such as macrophages, indicative of bacterial infection: pus cells and evenly distributed red cells sometimes indicative of ulceration; and Charcot-leyden crystals, often correlated with amebiasis and other parasitic infections. The cellular exudate in dysenteric stools containing trophozoites of E. histolytica usually contains few, if any, pus cells. Red cells, if present, are likely to be in clumps.

Report ALL parasites and any other abnormal findings.

C. **Occult blood test:** The test should be made on every stool received in the laboratory. Ham's method, modified,[3] is recommended. It is an easy to perform filter paper technic using either Benzidine base or Benzidine dihydrochloride in a one per cent relatively stable solution.

Any stool specimen which is received in the laboratory with an order for an occult blood test only should also be described both macroscopically and microscopically. Unsuspected parasitism is often discovered as well as other abnormal findings of which the physician should be apprised.

D. **Culture for protozoa:** It has been said that culturing for protozoa is at the bottom of the scale as a diagnostic procedure. There are, however, definite advantages to be gained from it. A culture method used routinely gives the technologist one more aid in detecting an infection and it is especially valuable in finding Dientamoeba fragilis. This important organism for which there are no known cysts is often found in cultures from formed stools.

There are various culture media any one of which will give excellent results when properly used. It is wise to employ a single medium and gain experience with it rather than to use several. A liquid medium is well suited to the needs of the general clinical laboratory. Balamuth's liquid medium[2] is highly recommended. It is simple to use and supports the growth of all protozoa including the flagellates with the exception of Giardia lamblia.

Culturing from a mushy or semi-fluid stool is preferred, using about 0.5 c.c. of fecal material. The specimen must be as freshly

passed as possible. When necessary to culture from a formed specimen inoculate a small portion about the size of a pea and combine the material from the surface, crevices and the interior of the fecal mass. It requires experience to know just how much feces to use as there will be little or no growth either from too little or from too great an inoculum.

Examine microscopically in 24 to 48 hours by removing a small amount of material by capillary pipette from the *top surface* of the sediment to a drop of saline on a slide. Allow time for the amoebae to settle and become active.

E. **Permanent stains:** Whenever protozoa are found the busy laboratory will find it advantageous to make permanent stains which may be studied at a time when the pressure of work lessens. It may happen, moreover, that only one species is observed in wet mount. Its presence indicates that the patient has been in contact with contaminated material harboring the organism and always suggests the possibility of multiple infection. Permanent stains not only increase the chances of finding other organisms but they are sometimes necessary diagnostically in order to study nuclei; they may be examined when time permits; they constitute a record; and, they furnish reference and teaching material.

There are excellent staining methods for which the literature or any textbook may be consulted. For general clinical laboratory use Markey, Culbertson and Giordano's[4] rapid method is most satisfactory.

F. **Concentration technics** (directions may be found in any standard text): At least one type of concentration procedure should be performed for each patient. Since each method offers its own advantages and disadvantages, it is sometimes necessary to use several of them. The method(s) employed will depend upon microscopic findings, the patient's symptoms, his travels, and the investigator's intuition.

1. The **formalin-ether** sedimentation method described by Ritchie[5] in 1948 is one of the best of the concentration procedures and is one which can almost claim to be all-purpose in function. It is excellent for the recovery of protozoan cysts and does not distort them. It is efficient for the recovery of ova, larvae and the fragile infertile Ascaris eggs. The sediment resulting from the process is preserved in formalin and may be examined when time permits.

2. The **zinc sulfate** flotation technic for the recovery of helminth ova and protozoan cysts is probably the most widely used. The method is efficient for all ova with the exception of the operculated ones, infertile Ascaris eggs and the Schistosomes. Larvae and some adults are recovered. Protozoan cysts are often distorted particularly those of the small race E. histolytica.

Satisfactory results have been obtained by the following modification of the method. Prepare a fine fecal suspension in lukewarm tap water and strain it through two layers of gauze into a 15 c.c. centrifuge tube. Spin for one minute at 2500 r.p.m. Pour off the supernatant. Resuspend the sediment in zinc sulfate of sp. gr. 1.20 (instead of sp. gr. 1.18). Fill the tube with zinc sulfate to one-half inch of the top. Centrifuge as above. Remove the tube carefully without agitation from the centifuge and stand upright in a rack. Add zinc sulfate to form a meniscus. Let stand 10 minutes then remove the surface film by touching it gently with a coverslip. Place the coverslip on a slide and examine microscopically.

The use of a coverslip to remove diagnostic material is preferred to that of a bacterial loop which tends to disturb surface tension, allowing eggs to fall or it misses them entirely.

3. The **brine flotation** technic reported by Willis in 1921 is practically specific for hookworm and whipworm although it is good for all ova other than the operculated ones and the Schistosomes. Strongyloides are destroyed.

4. **Sedimentation** for the recovery of parasites is particularly suitable for operculate and Schistosome ova and Strongyloides but the method may be used for the recovery of all ova, larvae and cysts. The method has the disadvantages of being time consuming and of not removing debris.

5. The **acid-ether** concentration technic (Mathieson and Stoll, 1945) is useful for the recovery of most helminth ova but is particularly recommended for the Schistosomes. Protozoan cysts are shrunken and unrecognizable. If Schistosomiasis is suspected, the material for the application of the test should be taken from the outer surface scrapings of the stool specimen.

The method may be modified by the use of a wetting agent to yield a greater number of ova. Triton NE is efficient for this purpose. To use, add 0.6 c.c. of a 10 per cent aqueous solution of Triton NE to the acid-ether mixture and mix. Continue with the regular procedure.

Other wetting agents include glycerine, Calgon and Drene. They should be used sparingly—only a few drops—so as not to distort the eggs.

G. **Anal swabs for pinworms:** Pinworm ova are rarely found in or on the feces but are obtained from the anal and perianal areas by means of the anal swab. An excellent and inexpensive swab can be made in the laboratory by placing cellophane, sticky side out, over one end of a tongue depressor. The free ends of the tape are anchored securely by binding with more tape or by a rubber band.

The swab is applied to the perianal area of the patient before he has bathed in the morning and before he has defecated. The

cellophane tape with adhering material is removed from the tongue depressor and is pressed smoothly, sticky side down, on to a glass slide A small amount of saline is run under the tape and the slide is then examined with the low power of the microscope for the adult worms or the eggs.

A convenient container for the swab is a large, cotton-plugged test tube.

CONCLUSION

The direct wet mount in saline is the single most important diagnostic method for finding intestinal parasites but this one procedure alone will not detect all infections. Last year in this laboratory which handles a large volume of work, which reports all parasites found and which uses the above mentioned supporting methods, 86 per cent of the infections reported were found initially in direct wet preparations. This figure includes the anal swabs on which pinworm ova were found. The remaining 14 per cent of the infections were found either in culture only (9 per cent) or not until one or more concentration methods had been performed (5 per cent).

One-half of the infections found in culture only were caused by Dientamoeba fragilis and the material cultured had come from formed stool specimens.

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THE GAVEL

With this New Year, let us take the time to decide what kind of professional advancement each of us wants. Naturally, security is not to be taken for granted, but where are we gaining confidence and respect. Such recognition comes from good public relations. Every member can be proud that achievement is being rewarded, not only in our own self respect, but within the last few months our exhibitors have given us a new opportunity to pick out those who personify medical technology. Read your NEWS RELEASE for those details that concern the things you want to know about your professional organization—what are you doing to get the most out of your society? Did you send in the questionnaires? The NEWS RELEASE has the explanation why we need your cooperation in these matters. Contribute your thoughts to achieve the place you want to be. Take part in your society locally, statewise, and ASMT will become an integral part of your way of life. March 15th is the deadline for all program contributions, thus "SERVICE THRU COOPERATION" will be the goal for the First North American Conference of Medical Laboratory Technologists, June 17-22, 1956 in QUEBEC CITY, CANADA.

Barbara Isbell, MT (ASCP)
President, A.S.M.T.

EXPERIENCE WITH HYCEL HORMONE CHEMISTRY SYSTEM FOR PROTEIN BOUND IODINE DETERMINATIONS*

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Since the publication of Chaney's[1] original article in 1940 on the determination of protein bound iodine, an extensive literature has developed.

It has been shown that in a normal individual the function of the thyroid gland is remarkably constant. Deviations from the normal can be of all degrees and even very slight, or subclinical variations can be demonstrated by the PBI.

About 98% of all serum protein bound iodine is derived from thyroxin, the hormone produced by the thyroid gland, so for all practical purposes the PBI is a direct measure of the functions of the thyroid gland.

The literature has been of two main types, clinical and technical. The large number of clinical publications have established the unquestioned superiority of these determinations in diagnosing thyroid malfunction. Of perhaps greater importance has been the unique role of protein bound iodine determinations in following and guiding therapy.

However, the clinical value, as in all laboratory determinations depends on the accuracy of the determination. To date, several different methods have been proposed, the most important of which are Chaney's acid distillation,[1] Salter's alkaline-ashing[2] and Zaks perchloric-oxidation.[3] Both the alkaline ashing and perchloric oxidation procedures are time consuming, subject to many sources of error and limited by indefinite end-points. On the other hand, the acid-distillation method presents a short and fairly simple analysis with few sources of error. Since the appearance of Moran's[4] excellent paper on the factors affecting this determination it is the method of choice.

The modified method is a comparatively simple procedure which can be easily performed by a competent technologist. This is a marked advance, for it removes the protein bound iodine determination from the realm of the difficult and unusual and places it as a routine procedure in the clinical laboratory.

The method consists of four well defined parts. Part One consists of precipitation and washing the proteins. Part Two destroys the proteins by acid digestion and simultaneously oxidized the freed iodine. In Part Three the iodine is reduced and distilled by reflux distillation into alkali. In Part Four the catalytic action

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of an aliquot of the distillate is compared in its action on the ceric sulfate—arsenious acid reaction with a group of standards. The entire procedure can be performed in approximately one hour, while twenty or more determinations can be performed in an eight hour working day.

Since the acid-distillation method is essentially a closed procedure many bugaboos can be immediately dispelled. It does *not* require a special uncontaminated room with filtered air system. The analyst does not need a doctorate in order to be competent. Exercising reasonable care in keeping reagents and equipment free of iodine or mercurial compounds is all that is required.

I would suggest that anyone starting out on PBI determinations use all new glassware. These should include centrifuge tubes, pipettes, cuvettes and even glass beads. This glassware should be reserved for the use on protein bound iodine determination and should be washed and stored separately from other laboratory equipment.

Ordinary distilled water is usually sufficiently pure for the determination. If not, passage of water through a simple ion-exchange resin will purify it. Pipettes and cuvettes are adequately cleaned by immersion in a strong detergent solution. The digestion flasks are cleaned by simple rinsing with a strong sodium hydroxide solution followed by several rinsings with distilled water. We use distilled water generously in cleaning all glassware.

Since iodine contamination obviously would increase the values obtained, and mercury inhibits the reaction, the only other care that need be taken is to be sure that a Van Slyke apparatus does not flank one side of the determination area and a Gram-stain rack flank the other side.

The analyst is now faced with only one problem and that is the preparation, purification and testing of the reagents. Moran's[1] paper gives an extensive list of tests by which the prepared reagents may be analyzed for suitability and some procedures by which they may be purified. This preparation is long and arduous and is the real reason so many analysts have been unable to perform the determination successfully. Fortunately, the recent appearance of HYCEL reagents has removed that obstacle and laboratories may now purchase these reagents ready for use. This problem of pure reagents, so happily solved for the acid-distillation method, still applies to the alkaline-ashing and perchloric-oxidation methods. In addition, the preparation of perchloric acid should be undertaken with the same degree of spirit that one would undertake the compounding of nitroglycerine. Both are extreme explosives.

The method which I have successfully employed is based on

Moran's work and is as follows: To 3 ml. of serum in a 40 ml. centrifuge tube are added 30 ml. of distilled water, 2.5 ml. of 10% sodium tungstate and 2.5 ml. of 2/3 N. sulfuric acid. The precipitate is mixed with applicator sticks, centrifuged a few minutes and the supernatant is decanted. The precipitate is re-suspended in 35 ml. of water, centrifuged again and the supernatant is discarded. The precipitate is dissolved and transferred to a 500 ml. 2-neck boiling flask employing 40 ml. of 70% sulfuric acid. To the flask are added several glass beads and 2.5 ml. of 60% chromic acid. The mixture is boiled for 3 minutes and allowed to cool for 3 to 5 minutes. 25 ml. of water are added and the mixture is boiled for eight minutes, employing a cone heater. After cooling for 5 minutes, 25 ml. of water are added to the flask and it is connected to the extraction apparatus which contains 1 ml. of 1% sodium hydroxide. The mixture is brought to a boil. When vigorous boiling starts, the dropping funnel is placed in to the side neck of the boiling flask and 10 ml. of 30% phosphorous acid, followed by 0.5 ml. of 3% hydrogen peroxide are blown into the flask. Extraction is continued for eight minutes. The distillate is collected in a graduated 15 ml. centrifuge tube containing 0.5 ml. of 0.064 N. arsenious acid in 0.6 N. sulfuric acid. The tube is cooled to room temperature and the volume is brought to 12 ml. with distilled water. A 4 ml. aliquot is added to a cuvette containing 1 ml. of the 0.064 N. arsenious acid. A blank tube consisting of 4 ml. of distilled water and 5 standard tubes equivalent to 4, 8, 12, 16, 20 micrograms per cent of iodine are prepared. Also included is a 4 ml. aliquot from a reagent blank determination. At 30 second intervals, exactly 1 ml. of 0.012 N. ceric sulfate in 1.6 N. sulfuric acid is added to each tube. The optical density of each tube is read at the end of twenty minutes in the 30 second intervals against water at 420 m.u. The blank and the standards are plotted on straight graph paper. The value for the unknown is read from the curve and from it is subtracted the value of the reagent blank. The result is the protein bound iodine concentration in micrograms percent.

While the method is simple, it cannot be carelessly performed. Since the quantities of iodine involved are so small, meticulous care to details of technique is required of the analyst. Glass beads used should be small—about 3 mm. in diameter—to insure smooth, even boiling. Colorimetric readings should be timed exactly to correspond with the time intervals at which the ceric ammonium sulfate is added to the cuvettes. Readings should be made on optical density at the prescribed wave length.

Suitability of distilled water may be checked by setting up a standard curve. This curve should be gently sloping in the form of a partial parabola. Any sharp turns or humps in the curve, should indicate contaminated water. Glassware contaminated

with mercury or other heavy metals would fail to react, while an immediate clearing of the standards would indicate iodine contamination.

All reagents used are stable at room temperature except the hydrogen peroxide which must be stored in the refrigerator. With these precautions in mind, the analyst can approach the test with utmost confidence.

By employing the Hycel System which includes the reagents, special equipment and the simple method, I have performed over three thousand accurate determinations without any appreciable difficulties. Of added interest is the fact that Hycel also furnishes a manual of interpretations which is most useful. For the occasional analysis which one may wish to have corroborated, they also furnish a technical service which includes free determinations.

Because of my personal experience I recommend the Hycel System to clinical laboratories contemplating the performance of protein bound iodine determinations and those laboratories that have unsuccessfully attempted their performance in the past.

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The Armed Forces Medical Library announces the publication in May of a *Bibliography of Medical Reviews*.

The bibliography, arranged by subject, will contain approximately 800 references to review articles in clinical and experimental medicine and allied fields which have appeared in the calendar year 1955.

Copies of the *Bibliography of Medical Reviews* will be available upon request to the Director, Armed Forces Medical Library, 7th Street and Independence Avenue, Southwest, Washington 25, D. C. in the order of receipt.

A SIMPLE METHOD FOR THE QUANTITATIVE DETERMINATION OF SUGAR IN URINE*

ALFRED S. GIORDANO, M.S., J. LON POPE, M.Sc., AND BERNADINE HAGAN, M.Sc., M.T. (A.S.C.P.)

INTRODUCTION

Approximately 50 years ago, Benedict[1,2] introduced into clinical pathology two methods, each using a specific reagent and designed to detect glucose in urine. One was known as Benedict's qualitative method, and the other, which was designed to measure the concentration of sugar in urine, was known as Benedict's quantitative method. During the ensuing years, many modifications have been proposed but there has been little deviation from the original procedures. Although these two techniques are widely used, there have been relatively few studies on the accuracy of either procedure.

Approximately 10 years ago, Kasper[3] reported a modified reagent tablet had been developed for the detection and measurement of urinary glucose. The reagent tablet is used in a simplified Clinitest tablet bought on the open market and manufactured by the Ames Laboratories of Elkhart, Indiana in which all of the ingredients for the test are contained in the tablet. When the tablet is added to a definite quantity of diluted urine, the ingredients react with the liberation of heat and if sugar is present, the blue cupric compounds are reduced to yellow or orange cuprous compounds. A color chart supplied with the reagent can be compared with the reaction mixture to indicate the approximate sugar concentration.

Recently, one of the authors (A.S.G.), participated in a comparative study[4] to determine the accuracy of this test and Benedict's qualitative test for the detection of sugar in urine. It was found that both qualitative tests were completely accurate when properly used. However, the Benedict's qualitative test is extremely sensitive and gives trace reactions with many urines from normal individuals. It was concluded that errors can be made with either method but were more likely to occur with Benedict's method due to improper heating or to its great sensitivity. Although this procedure may represent no obstacle to the well-trained technician, it may be poorly performed by ancillary personnel in the physician's office or by the patients who must test their own urine. That the simplification of testing procedures will aid in the elimination of errors is evident.

This simplified test not only demonstrates the presence or absence of urinary sugar, but a color chart supplied with the product enables one to quantitate the amount of sugar present. This

* Received for publication July, 1955.

report describes experiments designed to evaluate the accuracy of the tablet reagent and Benedict's quantitative test for the measurement of urine sugar concentration.

METHODS

The method employed by Benedict's quantitative procedure is the macromethod described in the 12th edition of Hawk, Oser and Summerson's "Practical Physiological Chemistry," which is very similar to the original description given by Benedict for the quantitative method, the principal difference being that the titration is carried out with diluted urine.

The procedure used for the new tablet reagent is essentially that described by the manufacturer in the direction sheet accompanying this product. These directions are as follows:

1. With dropper in upright position, place 5 drops of urine in test tube (15 mm. x 75 mm.). Add 10 drops of water.
2. Drop 1 tablet in test tube. Watch while reaction takes place. Do not shake test tube during reaction nor for 15 seconds after boiling has stopped.
3. After 15 seconds waiting period, shake test tube gently and compare with color scale.

INTERPRETATION OF TEST

Negative—the fluid will be blue at the end of a waiting period of 15 seconds.

Positive—if the urine contains sugar, the fluid will change color from blue to green to tan and finally through orange to brown, depending on the amount of sugar present. The shade of color is compared with a color chart to obtain the appropriate amount of sugar present.

In this study, all readings were made using daylight. The tube and color chart were held vertically in such a position that the light from a window entered from the side or back of the observer. A slight alteration in procedure which we employed was that we did not shake the tube at the end of the 15 second waiting period. Also, in part of the work, 0.25 ml. of urine and 0.50 ml. of water were used instead of 5 and 10 drops. If the glucose reading on the first test was between 0% and 1%, this reading was employed as the sugar concentration. However, if a sugar concentration of over 1% and less than 2% (+++ to ++++ in the first test) was obtained, the test was repeated on a dilution of 1 volume of unknown urine with 2 volumes of a normal sugar-free urine. The result of this second test was then multiplied by 3 to obtain the sugar concentration. If the glucose concentration was over 2% (more than ++++ in the first test), the test was repeated on a dilution of 1 volume of unknown urine with 9 volumes of a normal sugar-free urine. The results of this reaction on the 1 : 10 diluted urine was then multiplied by 10 to obtain the glucose concentration.

EXPERIMENTAL

The color chart provided by the manufacturer has 6 colors that are designated as follows:

Negative	0%
Trace	¼%
+	½%
++	¾%
+++	1%
++++	2%

In our study, we have employed these colors and values but have interpolated intermediary colors between the stated concentrations and thus have assigned intermediary concentration values. It should be stressed that this interpolation involves familiarity of the operator with the reaction along with a good sense of color differentiation. The interpolation is particularly useful in the determination of sugar concentrations of over 1%, after dilution with normal sugar-free urine.

In order to evaluate the three methods, studies were made with urines which gave a negative reaction with the tablet reagent. The results obtained with 9 such urines are shown in Table I.

Table I
Glucose Values on Negative Urines

No. Urine Sample	Benedict's Quantitative Gm %	Tablet Reagent Test
1. Neg. urine—Patient	0.07	0.0
2. Neg. urine—Patient	0.15	0.0
3. Neg. urine—Patient	0.12	0.0
4. Neg. urine—Patient	0.07	0.0
5. Neg. urine—Patient	0.08	0.0
6. Normal subject	0.34	0.0
7. Normal subject	0.23	0.0
8. Normal subject	0.17	0.0
9. Normal subject	0.16	0.0

It will be seen that each urine gave an apparent sugar concentration with Benedict's quantitative method. Additional studies were carried out with negative urines to which known quantities of glucose were added. A typical sample of the results with these measurements are shown in Table II. Results with Benedict's quantitative test and the tablet reagent test show deviations from actual values of the same order of magnitude. The results with the tablet indicate that it is a suitable procedure for the quantitation of glucose in sugar-containing urine samples.

The suitability of the technique for measuring urine sugar concentration with the tablet reagent is also attested to by the procedure which we employ with urines giving a 3+ or 4+ reaction. With such urines, it is our practice to set up different dilutions on the specimen. Each dilution is read against the color chart. We have found that values obtained on different dilutions check

quite well. All such dilutions are made with urines that do not contain sugar, *since dilution with water will tend to give high results.*

Table II
Urine Glucose Values on Urines of Known Glucose Concentration
Specimens made by adding a measured amount of glucose to negative urines

Sample No.	Glucose Concentration Gm Percent	Benedict's Quantitative		Tablet Reagent Test	
		Conc.	Percent Error	Conc.	Percent Error
1.	0.8	1.07	+34	0.75	— 6
2.	1.6	1.80	+13	1.9	+19
3.	3.2	3.43	+ 7	3.1	— 3
4.	6.4	6.4	0	6.3	— 2
5.	0.7	0.8	+14	0.75	+ 7
6.	1.4	1.50	+ 7	1.7	+21
7.	2.8	2.93	+ 5	2.8	0
8.	5.6	5.5	— 2	6.3	+13
9.	0.9	1.02	+13	0.88	— 2
10.	1.8	1.99	+11	1.9	+ 6
11.	3.6	3.84	+ 7	3.8	+ 6
12.	7.2	7.4	+ 3	7.5	+ 4

An independent worker in a diabetic clinic laboratory has made quantitative measurements on more than 1500 urine samples which gave positive reactions with Benedict's qualitative reagent. Sugar concentration in these urines was determined in both Benedict's quantitative procedure and with the new tablet reagent. In no instance was there significant disagreement between the results obtained with the two methods. The magnitude of this series is such that one can safely state that the new tablet reagent is a satisfactory method for the quantitation of sugar in urine.

In the diabetic clinic laboratory as well as the general laboratory or the physician's office, the saving of time is of great importance. This is one of the advantages of the tablet reagent that is quite obvious. The careful titration of a urine using Benedict's quantitative method requires several minutes whereas several measurements can be made in one or two minutes with the tablet reagent. The disagreeable odors encountered in a laboratory where Benedict's quantitative determinations are being carried out are eliminated when the tablet reagent is used. The simplicity of the procedure is an advantage that is appreciated by all.

SUMMARY

A comparison has been made of Clinitest tablet and Benedict's quantitative method for the measurement of urinary sugar concentration. The accuracy of the Clinitest tablet reagent and Benedict's quantitative method was of the same order of magnitude except with the urine having low sugar concentration. With these urines, Benedict's quantitative procedure gave values above the true concentration. The simplicity, speed and accuracy of the Clinitest tablet reagent make it a desirable procedure for quantitation of urinary sugar.

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HELPFUL HINTS: FROM C. G. S. SCIENTIFIC PRODUCTS DIVISION, AMERICAN HOSPITAL SUPPLY CORPORATION

Here's a suggestion which may be new to you: On removing wrinkles from tissue sections. It involves a hot plate* and a technique you can master in a few seconds:

> "Often wrinkles which appear irremovable on the water bath at 44° C. disappear completely when sudden heat is applied by moving the slide *quickly* back and forth across the edge of the hot plate set at 100-150° F. The paraffin melts, allowing free positioning of the tissue section. Occasionally it may be necessary to use a testing needle to facilitate removal of wrinkles while the paraffin is melted. Once the wrinkles are removed, the paraffin may be solidified again by blowing lightly on the tissue section.
>
> ". . . this quick warming technique provides factual assurance that tissue sections will not wash off the slides when being carried through the various solutions in the staining process."

Here are some ideas about laboratory glassware that we think are worth saving.

1. A quick way to identify types of glass (when making repairs, constructing special apparatus, etc.)—Pyrex will not be visible when immersed in a solution of 16% methyl alcohol and 84% benzene. Kimble's N51A glass cannot be seen in a mixture of 8% methyl alcohol and 92% benzene. Common lime glass (soft glass) disappears in 25% carbon disulfide and 75% benzene.
2. To cope with the nuisance of a balky, frozen stopper or stopcock, just try this: immerse stopper in a solution of 10 parts chloral hydrate, 5 parts glycerine, 5 parts water, and 3 parts 25% hydrochloric acid.
3. And, perhaps you didn't know that poorly fitting stopcocks can frequently be seated properly by making a thick paste of 500 mesh (approximately) aluminum oxide or silicon carbide with water—apply layer to plug—insert in barrel and rotate back and forth through a three-quarter turn for a minute or two.

DIFFERENTIAL DIAGNOSIS OF ANEMIAS WHICH PRESENT PERIPHERAL LEUKOERYTHROBLASTOTIC REACTIONS

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A leukoerythroblastotic reaction can be defined as the presence of immature neutrophils and nucleated red cells in the circulating blood. Vaughan (1936)[1] is generally credited with the term "leuco-erythroblastic anemia," but leukoerythroblastosis[2] or leukoerythroblastic reaction have come to be more popular terms because the anemia may be much less prominent than the leukoerythroblastosis. Still another way of describing this sometimes puzzling peripheral blood picture is "a leukemoid reaction with normoblasts." In any event a large variety of conditions can be associated with the presence of immature myeloid cells (almost always only immature neutrophils, not immature eosinophils or basophils) and nucleated erythrocytes in the circulating blood; the total numbers of either leukocytes or developing nucleated red cells is extremely variable, and variations in the degree and type of anemia are generally helpful in the consideration of diagnostic possibilities.

If the term leukoerythroblastotic reaction is to embrace even those cases in which a very occasional neutrophilic myelocyte and normoblast are seen in a blood film, then many anemias as well as other conditions require consideration. The problem of differential diagnosis might be clarified by initial listing and subsequent discussion of the categories which deserve consideration.

Leukoerythroblastotic reactions may occur in the following conditions:

1. Anemia of acute blood loss.
2. Severe hypochromic anemias.
3. Hemolytic anemias.
   a. Familial hemolytic anemia.
   b. Acquired hemolytic anemias.
   c. Erythroblastosis fetalis.
   d. Sickle cell anemias and anemias due to other abnormal hemoglobins.
4. Megaloblastic anemias.
5. Myelophthisic anemias.
   a. Leukemia and non-leukemic malignant lymphomata.
   b. Metastatic tumor.
   c. Multiple myeloma.
   d. Gaucher's disease.

6. Aleukemic myelosis (myeloid megakaryocytic hepatosplenomegaly, agnogenic myeloid metaplasia, atypical myelogenous leukemia).
7. Polycythemia vera.
8. Exogenous hemochromatosis with refractory anemia.
9. Other conditions including severe infections, cardiac failure, uremia.

Acute Blood Loss

Generally the anemias of acute blood loss offer no serious problem in diagnosis because unless blood loss is internal, one is generally aware of its occurrence, and often the leukoerythroblastotic reaction is not discovered because there is no real necessity for daily morphologic studies. One source of confusion is the blood loss which occurs with thrombocytopenia. This may be sufficiently great to be attended by a noticeable leukoerythroblastotic reaction, and the latter may provide undue false emphasis on the possibility of leukemia.

Hypochromic Anemias

That severe hypochromic anemias based solely on severe iron deficiency, can be attended by leukoerythroblastotic reactions was shown experimentally by Hamre in 1947.[3] In the severe iron deficiency anemia of infancy, it is not at all uncommon to find normoblasts in the blood; immature neutrophils and varying degrees of thrombocytosis may also occur. These findings along with the pallor and hepatosplenomegaly often bring forth the clinical concept of leukemia. One should be aware of the frequency of this pattern in the severe iron deficiency anemias of infancy and feel relatively secure that leukemia in infancy almost never presents with this picture. Aspiration biopsy of the marrow is generally unnecessary.

In the hypochromic anemias of chronic blood loss, leukoerythroblastotic reactions are relatively uncommon. If it is well established that chronic blood loss is the cause of the anemia, that there has been no recent acute blood loss to explain the picture, and that bleeding is from the gastro-intestinal tract, aspiration of the marrow should be helpful in attempting to determine whether or not metastatic tumor or some other space-occupying lesion might be responsible for leukoerythroblastotic reaction.

Hemolytic Anemias

Hemolytic anemias can of course be accompanied by remarkable normoblastoses and, to a lesser extent, by the presence of immature neutrophils in the blood. In these anemias, however, the normoblastosis is often *not* out of proportion to the degree of anemia, and the amount of polychromasia (or degree of reticulocytosis) seen in the peripheral blood. Here the reticulocytosis points to the rapidity of regeneration and/or release of erythro-

cytes into the blood stream, and under these circumstances the normoblastosis seems a part of the general regenerative phenomenon. Striking normoblastoses are common in erythroblastosis fetalis, sickle cell anemia, and Thalassemia major. Occasionally, with acquired hemolytic anemias, the peripheral normoblastoses are sufficiently great to cause one to consider the possibility of erythroleukemia. In these cases the lack of profound abnormalities in erythropoiesis should lead one away from this diagnostic possibility. In all of these hemolytic anemias except, possibly, erythroblastosis fetalis, there is outstanding normoblastic hyperplasia of the marrow; evidence of increased phagocytosis of erythrocytes is present; and varying degrees of hemosiderosis are found. The few marrows I have seen from infants with erythroblastosis fetalis have not shown outstanding normoblastosis, and I have felt that possibly the spleen or liver were more important erythropoietic organs in these cases.

In the so-called "aplastic crisis" of familial hemolytic anemia described by Owren[4] and the pancytopenic hypersplenic crisis described by Dameshek and Bloom,[5] normoblasts and reticulocytes may be profoundly diminished in the marrow and blood. When the regenerative phase occurs, the peripheral leukoerythroblastotic reaction may be startling in its magnitude.

Megaloblastic Anemias

It has been known for many years that megaloblastic anemias may show normoblasts, megaloblasts, and immature neutrophils in the circulating blood. Any of the types of megaloblastic anemia can exhibit this peripheral blood pattern. In our experience the megaloblastic anemias of infancy have shown the most remarkable leukoerythroblastotic reactions. When I was faced with the diagnosis of the first megaloblastic anemia of infancy, I felt that it probably was a form of leukemia involving primarily the red cells, but the accessory changes were so similar to those of pernicious anemia that I asked to have it treated as if it were a form of pernicious anemia. This case was reported by Aldrich and Nelson (1947)[6] along with additional cases of megaloblastic anemia. May's interest in the mechanism responsible for megaloblastosis culminated in a comprehensive study of the megaloblastic anemia of infants[7] and in the production of a similar anemia in monkeys.[8-11]

Myelophthisic Anemias

The term leukoerythroblastotic reaction is most generally used with reference to the so-called myelophthisic anemias or those anemias brought about by space-occupying lesions of the marrow. It is in these diseases that the finding of occasional normoblasts and occasional immature neutrophils in the blood is of utmost diagnostic import. Leukemia itself is a common cause of this pattern, and often the leukoerythroblastotic reaction is min-

imal. Here, our experience has been that subacute and acute lymphatic leukemias as well as the non-leukemic malignant lymphomata often show this pattern.

Metastatic tumor in the marrow has also been a common cause of leukoerythroblastotic reaction, but it has also been apparent that leukoerythroblastotic reaction does not accompany all instances of metastases to the marrow. Normoblastosis may be outstanding when anemia is relatively mild in metastatic, prostatic and mammary carcinoma. In other instances osseous lesions are easily visible in X-rays, and no hint of this is found in the blood. Leukoerythroblastotic reaction with mild anemia and mild reticulocytosis is of most value in metastatic carcinoma of the gastro-intestinal tract or bronchogenic carcinoma where osseous lesions may not be demonstrable or where only diffuse osteoporosis is found on roentgenographic study. In these cases tumor cells are almost always found on aspiration biopsy. Rohr[12] and Moeschlin[13] think that the normoblasts and immature granulocytes found in the blood in these reactions are derived from areas of myeloid metaplasia, particularly in the spleen, and that these immature cells are not, so to speak, forced out of the marrow by a space-occupying lesion. The validity of this concept is, of course, difficult to prove but in Rohr and Hegglin's[14] early description of the finding of tumor cells in the marrow, they noted splenomegaly in five of their ten patients with metastatic carcinoma of the marow.

Our files include 43 cases in which metastatic carcinoma has been found in aspirated marrow. Of these: 24 (56%) showed leukoerythroblastotic reactions; 3 (7%) showed normoblasts but no immature neutrophils in the blood: 4 (9%) showed immature neutrophils but no normoblasts in the blood; 9 (21%) showed *neither* normoblasts nor immature neutrophils in the blood; and on 3 (7%) no data on the blood are available. In 5 cases there was a peripheral leukoerythroblastosis and tumor was known to be present in the marrow, but this was not demonstrable on aspiration biopsy. Two of these were mammary carcinoma; one was prostatic carcinoma; one was adenocarcinoma of the rectum; and information is incomplete concerning the fifth. One patient with metastatic mammary carcinoma showed peripheral normoblastosis, and we failed to demonstrate tumor cells on aspiration biopsy. Our files also contain the negative results of 52 aspiration biopsies done because the possibility of metastatic tumor was suspected. The blood from these patients did not show a leukoerythroblastotic reaction. The leukoerythroblastotic reaction seems an excellent index of possible involvement of the marrow with metastatic carcinoma, but it is to be noted that 21% of our positive cases did not show leukoerythroblastosis.

Leukoerythroblastotic reactions occur with multiple myeloma

but much less frequently than with metastatic carcinoma. In 44 cases of multiple myeloma in our files, leukoerythroblastotic reactions were recorded in 7 (16%); normoblasts were recorded in the blood in an additional 4 (9%); and immature neutrophils, but no normoblasts were found in 17 (39%).

In the five cases of Gaucher's disease I have seen: 2 showed peripheral leukoerythroblastotic reactions; 1 did not show normoblasts or immature neutrophils in the blood; and in 2 no blood studies are available. It is difficult to draw any conclusions regarding the frequency of leukoerythroblastosis from this series.

Aleukemic Myelosis

The disease aleukemic myelosis which holds such a controversial spot in the literature because of its multiple seemingly somewhat dissociated findings should be well-understood by morphologists for it can be very confusing clinically. Because of the remarkable abnormalities in megakaryocytes and platelets as well as the abnormalities in erythropoiesis, I have never been able to believe that it could be anything other than a variant of chronic myelogenous leukemia or of polycythemia vera.[15] Dameshek (1951)[16] provided one solution to the confusion by suggesting that the term "myeloproliferative disorders" might be used to embrace chronic granulocytic leukemia, polycythemia vera, idiopathic or agnogenic myeloid metaplasia of the spleen, megakaryocytic leukemia and erythroleukemia (including Di Guglielmo's syndrome). The literature contains many reports both for[15-19] and against[20-22] the idea that this disease is a form of leukemia. Downey[23, 24] has entertained both concepts. Recently Upton and Furth (1955)[25] have reported the transmission of a disease comparable to aleukemic myelosis in mice by a virus-like agent of low infectivity. They offer no claim that this is proof of the neoplastic character of the disease, but they do state that in the early stages of the disease erythropoietic hyperplasia occurs in the spleen in the absence of detectable aplasia of the bone marrow. In other words, the myelofibrosis of the marrow does not seem to be the initial process, and the remarkable erythropoiesis in the spleen does not seem to be a compensatory phenomenon as many of the advocates of the non-leukemic nature of this syndrome imply.

Despite the controversy regarding the nature of the disease, the changes seen in the peripheral blood fall within a relatively specific range. Routine blood studies will generally reveal a mild normochromic anemia, a leukocyte count that is within or close to the normal range, and a differential count that is not particularly abnormal or unusual. The sedimentation rate may be slightly elevated. Platelets are usually normal or only moderately decreased. Red cell indices usually show no striking abnormalities unless there has been bleeding. In the latter case,

the anemia may be hypochromic. Frequently, in a routine differential count, normoblasts will be encountered and possibly the anisocytosis, poikilocytosis, and minimal to moderate polychromasia will receive attention. The latter findings are of cardinal importance. The changes in the erythrocytes are often sufficiently unusual to bring about further examination of the blood film. With this type of examination one will often come upon a very occasional cell containing coarse basophilic stippling (which usually proves to be iron with the Prussian blue stain),[26] and generally one will notice atypicalities in the platelets comparable to those described by Downey[24] and others.[22, 27, 28] The atypicalities may be of sufficient magnitude to have deserved initial attention, but at times they are not outstanding, and the well-known high index of suspicion which should be provoked by the peculiar anemia is ultimately responsible for finding these atypical platelets. If atypical platelets are found, one can often also find cells of the megakaryocytic series. In many cases the cells of the megakaryocytic series will be dwarf forms; they are generally relatively rare, only 1 or 2 being found in a survey of several slides. During this survey of the slides one will generally encounter occasional immature neutrophils and often an occasional myeloblast. There may be a minimal increase in basophils, but it is almost never comparable to the increase seen in the classic cases of chronic myelogenous leukemia. In our experience the presence of atypical platelets and their precursors in the presence of a leukoerythroblastotic reaction suggests that the condition is probably not metastatic carcinoma or any form of malignant lymphoma other than myelogenous leukemia of some type. These findings are virtually indicative of aleukemic myelosis.

Bone marrow aspiration generally serves to clarify the problem. Occasionally marrow is difficult to aspirate. Often although the marrow may not be difficult to aspirate, only a very low M-E volume is observed on the centrifuged specimens. When the marrow picture of 10 of these cases was analyzed in 1950,[15] it was found that the fat content of the marrow averaged 0.2% and the myeloid-erythroid volume 1.4%. Normoblasts averaged 22.8%, neutrophils 59.7%, myeloblasts 1.6%, eosinophils 0.7%, basophils 0.5%, and lymphoid cells 14.7%. Megakaryocytes were decreased in all of these cases and macrophages were prominent. When films are made of a drop of marrow aspirated from patients with aleukemic myelosis, often these films are less cellular than the blood. In cases of this type we generally feel obligated to attempt aspiration from several sites including the sternum, the ilium, and the spinous process of one of the vertebra. In one case we were rewarded with a somewhat higher M-E volume from the fluid aspirated from the vertebral spinous process. Oc-

casionally we have done surgical biopsies on patients of this type and have found the characteristic "myelofibrotic" changes. I do not consider the latter type of biopsy necessary to diagnosis when the clinical and hematological findings are otherwise characteristic.

It is not within the range of this report to include the clinical evaluation of our cases. However, a few words regarding the general clinical picture might be helpful.

Clinically the disease aleukemic myelosis offers some difficulty in diagnosis. It occurs in both sexes and has been reported in all age ranges, but it seems most prevalent between the ages of 40 to 60.[21] If one excludes the cases which begin with a mild initial erythrocytosis, and as such are much like polycythemia vera, one can say that the commonest symptoms are weakness, fatigue, lassitude, and varying degrees of pallor and anemia. Often these are apparently not sufficient to necessitate medical advice and a common early complaint is pain or fullness in the left upper quadrant or pain in the lower left chest which the patient interprets as pleuritic. There may be vague gastrointestinal complaints or there may be frank diarrhea or constipation. A mild hemorrhagic tendency is frequently present and the patient may recall excessive bleeding from tooth extraction several years prior to the present illness. Although splenomegaly is not invariably present even when anemia is sufficient to cause symptoms, many patients are aware of a mass in their abdomen and have been told their spleen is enlarged (occasionally as long as 10 years previously). Some patients complain of deep bone pain initially; many develop pain as the disease progresses. Occasionally febrile episodes occur. A few patients complain of dyspnea.

Physical examination often reveals pallor, splenomegaly (which may be massive), hepatomegaly and occasional petechiae or ecchymoses. Usually both the spleen and liver are firm and non-tender, but occasionally the spleen may show areas of tenderness (previous infarcts). Many times the liver is barely palpable or not enlarged. Other times the liver is easily palpable and the spleen may not be demonstrably enlarged. Occasionally pallor is the only physical finding. Occasionally the combination of pallor and a sore tongue may be confusing. Rarely there is jaundice as well as splenomegaly. Still more rarely hematemesis may occur; in these instances the disease is generally confused with cirrhosis.

The differential diagnosis includes primarily leukemia, Hodgkin's disease, other forms of malignant lymphoma, cirrhosis, pernicious anemia, hemolytic anemia, or some form of refractory or aplastic anemia. Bone pain generally does not dominate the picture, and usually the possibility of metastatic carcinoma seems poor because of the massive splenomegaly. Patients often will

have tried a variety of hematinics with no benefit. Some will have had temporary subjective improvement with intramuscular liver or vitamin B_{12}.

Polycythemia Vera

We* have recently studied 2 cases of polycythemia vera which presented with anemia, remarkable thrombocytosis and leukoerythroblastotic reactions and seemed difficult diagnostic problems. This has succeeded in emphasizing the fact that anemia may be profound in polycythemia vera, and that the thrombocytosis should serve as the clue to the etiology of the disease. The first patient was a thin, pale, 62 year old white woman whose chief complaint was "low blood." She had had melena of varying degree but no lesion had been found in her gastrointestinal tract other than hemorrhoids. She had had a hemorrhoidectomy 2 years prior to admission at which time there had been excessive blood loss. She admitted to bruising easily, and she had had pruritis for approximately a month. She had had a low grade fever for 6 to 8 weeks, and she had always been nervous. In the hospital efforts to uncover a source of chromic blood loss were not successful, and essentially her physical examination revealed only pallor, minimal hepatosplenomegaly, and scratch marks on the back, shoulders, and buttocks. At the time of admission her initial laboratory data were as follows: Hb. 6.7 grams; Ht. 25%; RBC 2,780,000 per cu. mm.; reticulocytes 8.6%; WBC 46,200 per cu. mm. with N-79%, L-10%; M-4%, E-4%, and B-3%. Platelets were recorded as markedly increased, and occasional normoblasts were seen. The marrow was hyperplastic and showed almost no fat. The differential was normoblasts 18%; neutrophils and precursors 61.8%; eosinophils 2%; basophils 0.6%; and lymphoid cells 17.6%. Because occasional atypical platelets were found in the blood, I felt this might be a hyperplastic phase of an atypical myelogenous leukemia, but I felt that we did not have sufficient changes in the blood to establish a positive diagnosis. Sections of the marrow showed a pattern rather typically that of polycythemia vera.† Despite these sections I did not feel that I could distinguish between polycythemia vera and a possible hyperplastic phase of aleukemic myelosis. The patient was given 1100 cc. of blood, and this transfusion allowed her to hold her hemoglobin to the level of 10 to 11 grams for 3 months at which time her hemoglobin was 7.3 grams, and the general pattern was similar to that encountered at the time of admission. She was given packed erythrocytes on two separate occasions with a total of 750 cc. She responded poorly to

* Cases studied by Dr. Robert B. Howard and the author and presented at the Minnesota Society of Internal Medicine, May 21, 1955.

† This pattern is illustrated by Rohr[15] and it has been discussed by Block, Bethard, and Jacobson[27] and Block and Bethard.[28]

these, and 4½ weeks after the first transfusions of packed RBC her hemoglobin was 9 grams, her leukocyte count 16,500 per cu. mm. with N-87%; L-9%; M-2%; E-1%; B-1%, and her platelet count was 1,272,000 per cu. mm. Because it was obvious that the transfusions were not going to control the situation, and because I felt strongly that this could be a case of polycythemia vera, the patient was given 3 mc. P32 orally. Three weeks later her hemoglobin was 6.6 grams; her white count, essentially the same; and her platelet count, 992,000 per cu. mm. Because we knew that this patient responded poorly to transfusions, because she felt fairly well with a hemoglobin of 6.6 grams, and because the red cells were now markedly hypochromic, we decided to try simple oral iron therapy. The following week the hemoglobin was 8.2 grams, the reticulocytes, 4.9%, the leukocyte count, 9,950 per cu. mm. with N-86%; L-7%; M-4%; E-3%, and the platelet count, 504,000 per cu. mm. Seven weeks later the hemoglobin was 16.5 grams, the hematocrit 61%, the leukocyte count, 14,500 per cu. mm. with N-75%; L-19%; M-2%; E-2%; B-2%, and the platelet count, 289,000 per cu. mm. At this point we felt reasonably convinced that the patient had polycythemia vera, and iron therapy was stopped. Two weeks later the hematocrit was still 61% and venesection (500 cc.) was done. About 10 days after this she had a severe epistaxis, and when she returned to the clinic two weeks after venesection, her hemoglobin was 16 grams, her hematocrit, 52.5%, and her platelet count, within the normal range. To doubly insure the diagnosis, she was allowed to build her hemoglobin for another week, and the next values were: hemoglobin 17.4 grams, Ht. 59%, WBC 6,800 per cu. mm. with N-74%; L-13%; M-6%; E-5%; and B-2%. The platelet count had risen to 486,000 per cu. mm.

At this point we felt that the diagnosis was definitely established; (500 cc. of blood were withdrawn), and she was given 5 mc. P32 orally. With subsequent venesections and P32 she has been maintained in excellent hematologic control for 8 months.

The second patient* was a 64 year old woman who had a splenectomy 5 weeks after she had been in a bus accident at which time it was thought there might have been traumatic rupture of the spleen. The laboratory values at the time of the bone marrow biopsy (6 months after splenectomy) were: Hb. 8.2 grams; RBC 5,780,000 per cu. mm. WBC 44,900 per cu. mm. with N-87%; L-5%; M-7%; E-1%. The marrow was remarkably hyperplastic. Normoblasts and megakaryocytes were increased, and there were outstanding peripheral normoblastosis and thrombocytosis. Reasoning that although the peripheral blood pattern was undoubtedly influenced by the previous splenectomy, still the hyperplastic marrow was not attributable to the splenectomy, it was felt that this patient also had polycythemia vera. About

two weeks after this diagnosis had been made, the patient's hemoglobin fell to 5.6 grams. The reason for the fall was not apparent on the basis of studies for hemolysis, and blood loss did not seem to be sufficient to account for this drop. Transfusions were required because of the low hemoglobin, but 5 millicuries of P32 were given promptly and two months later the platelets which had been at the level of 3,061,000 per cu. mm. at the time of transfusions had fallen to 378,000 per cu. mm. and the hematocrit had risen to 57%. Only venesection was employed at this time and within six weeks the hematocrit had again risen to 57% and the platelet count to 1,168,000 per cu. mm. She was again given 5 millicuries of P32 and further venesections were done. Control seems to be much more difficult in this case, and it has been only 3 weeks since P32 was last given.

In both of these cases the patients apparently had had fairly numerous severe nosebleeds. In the first patient this history was elicited *only* after she had had a severe nosebleed at the time when she had become polycythemic. Then she admitted to having had these many times in the past, but she is still not clear as to when they began although she thinks it may have been some five years ago. The second patient admitted to having had nosebleeds prior to the bus accident. It is also known in the case of the second patient that the spleen was enlarged to below the umbilicus prior to splenectomy, and on the basis of changes in the spleen and in a liver biopsy taken at splenectomy the pathologist† had commented that the changes would be compatible with the diagnosis of polycythemia vera. Both of these patients had had boring bone pain.

These two cases have made us regard platelet counts in the range of 1 million as of probably great diagnostic value in the uncovering of obscure cases of polycythemia vera. In the first of these patients at least, the anemia seemed not to be controlled by transfusions and although we have not had adequate opportunity to check this, it seems that since the anemia did not respond to the iron contained in the transfused blood it would probably also not respond to oral iron in the presence of the remarkable thrombocytosis.

Exogenous Hemochromatosis with Refractory Anemia

There is still a condition which to me is even more interesting than these cases of aleukemic myelosis and of polycythemia vera with anemia and thrombocytosis. This is a condition which appears to be some type of refractory, possibly mildly hemolytic anemia which is associated with outstanding hemosiderosis.

Patients with the findings I shall attempt to describe ulti-

* I am grateful to Dr. A. Rusterholz for the privilege of studying this case.
† W. D. Stovall, M.D., Rhinelander, Wisconsin.

mately develop diabetic glucose tolerance curves, bronzing of the skin, and cirrhosis of the liver; they have the features necessary to a diagnosis of hemochromatosis.

In 1953 Goldish and Aufderheide[29] and Aufderheide, Horns, and Goldish[30] reviewed the literature on exogenous hemochromatosis and presented three cases of exogenous or secondary hemochromatosis. Other recent reviews are available.[31, 32] The case presented by Goldish and Aufderheide[29] was one of a patient who had had a chronic refractory hypochromic anemia for 22 years. Although the patient had had transfusions, they appeared to play an insignificant role in the pathogenesis of the hemochromatosis. It was thought that the repeated oral iron therapy might have accelerated the process. In one of the two cases presented by Aufderheide, Horns, and Goldish[30] the patient was thought to have chronic myelogenous leukemia as well as exogenous hemochromatosis. The peripheral leukocyte count was leukopenic with a leukoerythroblastotic reaction and 7% basophils. The marrow was hypercellular due to an increase in the myeloid series, and basophils were numerous. This case is mentioned because although it may well have been chronic myelogenous leukemia, still in our series of cases we have at times advanced the thought that the only reasonable explanation for the changes we saw seemed to be some type of myelogenous leukemia. As more cases have accumulated I have come to believe that although the pattern may be an extremely confusing one, it is probably secondary, and *not* leukemic. At least six cases of remarkable hemosiderosis and refractory anemia have been and are being studied at the University of Minnesota Hospitals. Four of these have had secondary hemochromatosis by which I mean they have had evidence of cirrhosis of the liver on the basis of biopsy or autopsy findings and they have had mild diabetes on the basis of clinical findings or they have had fibrosis of the pancreas demonstrated at autopsy. One is a patient who was splenectomized in 1946 and is still in reasonable health despite massive hepatomegaly, diabetes, and a serum iron of 245 micrograms percent. She maintains a hemoglobin of 11 to 12 grams. One is a patient who has remarkable hemosiderosis of the marrow, a hemoglobin of 10.8 grams, and a serum iron of 200 micrograms percent. She is not now diabetic, and the only alteration in liver function tests is a minimally elevated prothrombin time. The sixth patient is one who has only very recently come to our attention. She has a hemoglobin of 7.4 grams, a serum iron of 214 micrograms, percent and outstanding hemosiderosis of the marrow. The latter two patients have been treated with various hematinics with no remarkable relief, and they have each had transfusions but the number of transfusions has not been sufficient to account for the degree of hemosiderosis.

My purpose is not to describe these interesting cases in detail but to attempt to describe the blood and marrow pattern which has been confusing to me and which is sufficiently distinctive to be worthy of further documentation.

The blood picture might best be described as one which is similar to that of pernicious anemia, Thalassemia minor, and aleukemic myelosis. There is an anemia which is usually macrocytic and usually only mildly hypochromic. Normoblasts are often but not always found in the blood. Platelets have been within the normal range or only slightly decreased. The total leukocyte count has been normal or more usually at the lower limits of normal variation or decreased. The changes in the red cells are not entirely distinctive, but they are extremely interesting, and they often give the first clue to the diagnosis. There is generally at least moderate anisocytosis and poikilocytosis. Some of the macrocytes are large oval cells which are well-filled with hemoglobin. Occasionally target cells are seen. Some of the smaller cells are the elliptical, slightly pale cells often seen with hypochromic anemias. Spheroidocytes, most of which do not have as small a diameter as the spheroidocytes of familial hemolytic anemia, are also often present. Some of the erythrocytes will be found to contain a coarse type of basophilic stippling, and this generally proves to be iron with the Prussian blue stain. These siderocytes are usually remarkably distinct cells but the percentage of siderocytes in the peripheral blood is generally not high and in no way gives a clue to the degree of siderocytosis which may be found in the marrow.[33] If normoblasts are found, they often contain particulate iron and in some cases the normoblasts have been large and abnormal in appearance. Very occasionally atypical platelets may be found but in none of our cases have we been able to find dwarf megakaryocytes in the blood. The normal or leukopenic total leukocyte count is generally accompanied by a decrease in the total number of neutrophils and a relative lymphocytosis and monocytosis. There has frequently been a relative increase in basophils. Neutrophils have generally shown toxic changes with varying degrees of nuclear pyknosis; the granules of the neutrophils tend to take the stain poorly. Some of the changes in the neutrophils occasionally resemble those seen in the myelogenous leukemias; pernicious anemia neutrophils are not found. Varying percentages of immature neutrophils including promyelocytes and even occasional myeloblasts have been encountered. The marrows from these patients have been routinely hyperplastic and normoblasts have comprised approximately half or more of the nucleated marrow cells. Variations in the morphology of the normoblasts in the marrow include macronormoblastosis, changes in occasional cells which cause them to resemble megaloblasts, and changes in

some of the cells which make them resemble the type of developing erythrocyte often seen in the so-called erythroleukemia. Megakaryocytes are variable in numbers, and in general are not profoundly altered either in the direction of increase or decrease. Cells of the granulocytic series do not show changes which allow a conclusive diagnos s of leukemia. Lymphoid cells are variable in percentage, but there is always a remarkable increase in macrophages. The latter may contain more phagocytosed erythrocytes than one normally sees, and an increased amount of pigment, much of which stains for iron, is obvious. With the Prussian blue stain it can be found that normoblasts in any stage of development may contain particulate iron, this finding being most pronounced in the late polychromatic and orthochromatic stages. Siderocytes are numerous in the marrow. Neutrophils often contain fairly large masses of hemosiderin, and monocytes as well as the macrophages which appear to be of reticuloendothelial origin also contain this pigment. This pattern is obvious in films of the marrow as well as in sections. In addition to these changes there may be a perivascular plasmocytosis and tissue mast cells have also been found. Sections of the marrow from these patients have been important in the separation of this condition from leukemia. Although the marrow is markedly hyperplastic, fat cells remain, and the fundamental marrow architecture is not seriously disturbed. Instead there is a remarkable normoblastic hyperplasia and hemosiderosis. In three of the patients granulomata have been found in sections of the marrow.[34] These granulomata have differed from those seen in tuberculosis, brucellosis, and sarcoidosis in that the epithelioid cells of the granulomata have contained iron. The meaning of the latter finding is not yet clear, but the finding is of great interest. Oddly enough, as reported previously, the granulomata do not seem to have formed as foreign body reactions to the iron. Instead, the iron seems to be incorporated within or upon the surfaces of the epithelioid cells. Further work on the possible meaning of these granulomata is in progress.

This report would not be complete, if I did not call attention to a relatively recent survey[35] on the importance of normoblasts in the peripheral blood. In this study of 1,496 cases, normoblasts were found in the blood in cardiac conditions, infections, cerebrovascular accidents, diabetes, etc. as well as in conditions known to cause profound changes in the marrow. Their presence was considered a poor prognostic sign. It should be emphasized that normoblasts and immature neutrophils can be found in the blood in diseases which are not of primary interest to hematologists. In our studies uremia, very severe infections and sepsis, and cardiac conditions have been the most frequent in this group.

Conclusion

It should be apparent that a leukoerythroblastotic reaction can occur in a wide variety of conditions. Of most interest to the morphologist are the leukoerythroblastotic reactions which occur with myelophthisic anemias, aleukemic myelosis, polycythemia vera, and the type of refractory anemia and hemosiderosis just described which is believed to be an initial stage in the development of secondary or exogenous hemochromatosis.

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HUMAN RABIES—DIAGNOSIS OF OBSCURE CASES*

GEORGE H. HAUSER, M.D. AND LUCILLE GODELFER, M.T. (ASCP)

To the medical technologist in a public health laboratory, the diagnosis of rabies is a routine procedure. Since the incidence of rabies in humans is less than 1 per 100,000 population, the probability that a medical technologist working in a hospital or private laboratory will be called upon to perform an examination for diagnosis of rabies is slight. However, your attention is called to a review of three obscure cases in which the etiologic agent would not have been detected if facilities had not been available for the laboratory diagnosis of rabies.

The three cases presented in this paper were diagnosed through facilities offered by the Division of Laboratories of the Louisiana State Department of Health. None gave a history of having been bitten by a rabid animal, the clinical diagnosis of rabies was not made during the course of the illness, and the possibility of death from rabies seemed remote.

CASE I

R. M., a 45-year-old colored male, complained of severe headache and took many aspirin tablets to relieve the pain without success. The following day attempting to relieve the headache, he took more aspirin and began drinking whiskey. He was able to continue working during these two days, but on the third day consulted a physician. Whether or not any diagnosis was made is unknown. After the visit to the physician the patient returned home and began to act so queerly that the family in alarm called the police. The patient hid under the bed and it was necesary for the police to pull him out. During the process, three policemen were bitten by the patient who was then placed in the city jail where he remained for two days. On the second day he died. An autopsy was performed and the brain sent to the State Laboratory for examination. The brain tissue was received in formalin and it was impossbile to make satisfactory direct microscopic smear or impression examination or to do a mouse inoculation test. Paraffin sections were made of the brain tissue, and microscopic examination of the stained sections showed Negri bodies and the case diagnosed as Rabies.

Case II—A white woman whose age was judged to be in the early thirties; the only clinical information obtainable was that the patient had died on or about the fourth day of an illness characterized in part by nervousness, excitability and possibly convulsions. At the time of death autopsy was not performed and the patient was embalmed and buried. Because of the un-

* From the Division of Laboratories, Louisiana State Department of Health, New Orleans, Louisiana.

certainty as to the cause of death, the body was later exhumed and brought into one of our State hospitals for post-mortem examination.

The pathologist's[1] report of the autopsy stated that "the skin, except at the right wrist was in excellent condition. At this location, there was a fair sized area showing maceration and brownish discoloration. A bluish discoloration was also noted in the skin overlying the right thenar eminence. However, section through the area showed no gross change in the muscle. The soft tissue of the fingers was quite hard (mummified) but not discolored, and on the right index finger there was a linear bluish discolored area measuring about 2 cm in width and 1 cm in length. There was no ulceration and no definite scar." Whether or not this was residual to an animal bite could not be determined.

From the gross examination, no definite cause of death could be determined. Microscopic examination of sections of the brain showed an acute polio-encephalitis which was believed to be of viral etiology. Rabies and other neurotropic diseases were considered, but in the routine hematoxylin-eosin stains no Negri bodies were demonstrated. Sections of brain tissue were then sent to the State Laboratory for further examination.

Due to the fact that the body had been embalmed with fixation of tissue, it was impossible to make direct microscopical examination or to do a mouse inoculation test. However, paraffin sections were made of the brain tissue and microscopic examination of the stained sections showed the presence of Negri bodies.

Because of the unusual nature of these cases, sections of both of the brains were forwarded to the National Institute of Health for examination and our findings were confirmed.

CASE III

J. H., a 30-year-old white male was admitted to a hospital and expired thirty minutes later. According to relatives, the patient had complained of a feeling of constriction in the throat for several days, associated with excess salivation. For twelve hours prior to arrival at the hospital he occasionally spat up small amounts of brown material. The night prior to admission the patient had received an injection by his local physician (believed to be morphine). The patient's wife stated that approximately one hour prior to his arrival at the hospital he went into a hypermaniac state, thrashing about wildy. At the time of admission to the hospital, the admitting officer gave him 7½ grains of sodium amytal and about twenty minutes later the patient became quiet. According to doctors on duty, it was impossible to examine the patient. However, they did observe that the patient's pupils were dilated and his pulse weak. At no time could his blood pressure be obtained. Thirty minutes after

admission the patient ceased breathing and was pronounced dead. It was noted that the patient was frothing at the mouth.

There were numerous conflicting stories about the history of the case. However, it was revealed that the patient was a hunter and had spent a good deal of time in the woods. There was a possible history of the patient's having eaten toadstools, and it was at first believed that either food poisoning, or some other type of poison might account for his illness.

Complete autopsy including brain showed no gross pathological findings. As some type of poisoning was suspected, contents of the stomach and organs were removed and prepared to be sent to a chemical laboratory for analysis. As the possibility of rabies was not considered at time of autopsy, portions of brain tissue removed were placed in formalin for routine microscopic examination. Shortly thereafter, rabies was considered a possible etiologic agent, and a small portion of brain tissue was removed from the formalin, washed in saline and placed in the ice box.

The following morning a messenger brought the brain tissue to the laboratory requesting an examination for rabies. The tissue received was a small piece of cerebral cortex. History of the case or knowledge of the condition of the brain tissue was not received at the time the specimen was submitted for examination. Consequently, routine procedure for examination of brain tissue for rabies was done. Small portions of the cerebral cortex were placed on slides and smear preparations made, stained, and examined microscopically. Several inclusion bodies which structurally resembled Negri bodies, but stained feebly were found. After careful study of the bodies, we were convinced beyond doubt that they were Negri bodies and that rabies had been the cause of death. However, to confirm our findings four Swiss mice were injected intracerebrally with a suspension of the brain material.

A preliminary report of our findings was then given by telephone to the pathologist-in-charge. During conversation with him, it was then learned that the brain tissue had been immersed in formalin. This possibly accounted for the feeble staining of the Negri bodies. There was doubt as to whether the mouse inoculation test would prove satisfactory.

However, the formalin had no effect on the virus, for on the seventh day after inoculation, the mice began to show definite symptoms of rabies, such as roughness of hair and tremors. On the ninth day, two of the mice died and stained smears of brain tissue showed numerous typical Negri bodies. The other two mice were paralyzed, so they were sacrificed and microscopic examination of their brain tissue also showed numerous typical Negri bodies.

From the foregoing case histories from patients who have died from rabies, we are reminded once again of the dreadful nature of the disease, and inevitable death. Fortunately, only a few individuals in the United States die each year of rabies, so this unpleasant picture is not too often brought to the public's attention. Two factors account for the low incidence of the disease in man namely (1) man's low susceptibility to the virus, only 10 to 20 per cent contracting the disease when bitten by a rabid animal, and (2) the administration of antirabic or Pasteur treatment.

Much work has been accomplished in the past few years towards producing safe and effective vaccine, yet it must not be assumed that it is either 100 per cent efficacious or harmless. Occasionally in overwhelming infections or in bites about the face, patients die before protection occurs. Complications following treatment with antirabic vaccine vary from slight local reactions such as redness, edema and tenderness to constitutional symptoms such as malaise, fever, paralysis and even death in a small percentage of cases. Sellers[2] states that antirabic vaccine is not harmless and it accounts for more deaths than rabies when given to persons only indirectly or remotely exposed.

Rabies is caused by a neurotropic virus. The diagnostic microscopic finding in the central nervous system is the "Negri body." Negri bodies are round or oval inclusion bodies, varying in diameter from 0.5 to 30 microns and are most commonly found within the cytoplasm of the large ganglion cells of the hippocampus major and in the Purkinje cells of the cerebellum. Special stains are used to demonstrate the bodies, the most universally used being Sellers'[3] stain. With this stain, the bodies stain magenta to purplish pink, with dark-blue to black basophilic inner bodies within the acidophilic background. The nerve cells are stained blue, and interstitial tissue pink.

The finding of typical "Negri bodies" within the nerve cells is considered conclusive evidence of rabies. However, there are times when the bodies do not develop and animal inoculation is necessary to establish the nature of the disease.

The public health laboratory is often called upon to assist in the diagnosis of rabies in man because it is set up for the routine examination of this disease in animals. Rabies in animals constitutes a major public health problem. Contrary to popular belief the disease is not confined to any particular section but has appeared in every state in the United States. Consequently, every public health laboratory has trained personnel in this field or assistance may be obtained through the United States Public Health Service. In our State, laboratory facilities for the diagnosis of rabies are made available in the Central Laboratory in New Orleans and in three of our regional laboratories.

All animals are susceptible to rabies but epidemiologically members of the canine family, especially the dog, play the most important role in spreading the disease. Nearly 90 per cent of human cases arise from this source.

The clinical diagnosis in animals is sometimes very characteristic but must not be relied on, for often other diseases may simulate it. Definite diagnosis can only be established by laboratory tests and the most accurate and reliable methods at the present time are (1) demonstration of "Negri bodies" in the brain tissue and (2) mouse inoculation test.

As technologists, all should be interested in diagnostic laboratory procedures so it might be apropos to present briefly the methods used for laboratory diagnosis of rabies in animals which methods are also applicable to examination in man.

The brain of the suspected animal is removed as follows: a medial incision is made over the skull through the skin and muscle which are dissected laterally to expose the skull. By means of a chisel and hammer the skull is removed exposing the brain and the entire brain including the cerebellum is removed and placed on a paper picnic plate. The plate should be definitely labelled with the number of the specimen and the date. With a sharp scalpel the cerebellum is severed from the cerebrum and cut longitudinally. A clean slide is placed against the cut surface and pressed gently to obtain impression of the cerebellum tissue. In making impressions this way a large number of Purkinje cells are obtained on the slide. The hippocampus major (Ammon's Horn) is then dissected out and small bits of grey matter placed on a glass slide near one end. With a second slide, the tissue is flattened and a thin smear is made. Small pieces of cerebral cortex, hippocampus major and cerebellum are placed in 50% glycerol-saline solution and kept three months for later use if necessary.

The slides while still moist are immersed in Seller's stain contained in a Coplin jar and removed at once. They are rinsed with tap water and examined microscopically. Another satisfactory method is a modification of Williams[4] in which the smears while moist are fixed in methyl alcohol, defatted with ether and stained with a solution of basic fuchsin and methylene blue in water.

It takes an experienced technologist to find and identify "Negri bodies" in smears. There are other inclusion bodies resembling Negri bodies, that are produced by other viral diseases, especially in animals. At times, the Negri bodies are abundant, but again they may be very scarce and are found only after long and diligent search.

In our laboratory four slides each from the hippocampus and cerebellum are examined by at least two experienced technologists and if typical Negri bodies are found, a report is sent out

stating "animal brain smears positive rabies" and no further test performed unless other tests are specifically requested.

Failure to find the "Negri bodies" in direct smear examination does not preclude the possibility of rabies. Unfortunately, about 10 per cent of the brains negative by direct examination are found to be positive by animal inoculation. This is especially true if animals are killed in the early stages of the disease before the Negri bodies had time to develop or in cases of dumb rabies. Johnson[5] found that about 50 per cent of dogs infected experimentally with street virus developed the paralytic form of the disease and of these less than 50 percent showed Negri bodies.

The technic for the mouse inoculation test is simple. Grind small portions of the cerebral cortex, cerebellum and hippocampus in a sterile mortar, and dilute with sterile saline to make an approximate 10 per cent suspension. Let stand or centrifuge and inject 0.03 ml of the supernatant intracerebrally into each of four anaesthetized 3-4 week old white Swiss mice. Keep mice under observation for 30 days. If the virus is present in the brain tissue, the mice begin to show symptoms between the 7th to 10th day and usually die from the 9th to 12th day. Examination of smears from the brain tissue of the mice usually show typical Negri bodies. However, if no Negri bodies are found in smears from mouse brains showing classical systoms of the disease, mice are reinoculated with a suspension of these mouse brains. Occasionally, the bodies do not show up in the first passage, but will be found on second or subsequent animal passage.

SUMMARY

1. Three cases of human rabies were presented which showed outstanding neurological signs and symptoms. The clinical diagnosis in these cases was obscure.
2. Two of the cases were diagnosed by histological examination, and one by stained smears and mouse inoculation test.
3. Although stained smears and animal inoculation are the methods of choice for the laboratory diagnosis of rabies, histological section should not be overlooked when these other methods are not applicable.
4. It is therefore important to keep in mind and to consider the diagnosis of rabies in any human case dying with outstanding neurological symptoms, especially in a community where rabies is prevalent in animals.

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EDITORIAL

On a rather coolish evening, as Texas temperatures go in early December, instead of writing my usual Christmas notes to friends, I found myself thinking of the past year—of some of the events in which I was privileged to participate—of friends, old and new, whose company I had enjoyed—so, in this first issue of the Journal in 1956, I want to share with you some of these thoughts, however personal they may seem . . .

As summer was passing into autumn in 1955, I met Miss Elizabeth Pletscher, a medical technologist in Zurich, Switzerland. On the terrace of a restaurant overlooking beautiful Lake Zurich, we sat and talked for many hours—as old friends—talked about medical technologists, their problems, their aims and ideals. Elizabeth has done more than any one technologist to bring together representatives of our profession from all over the world. It is she who, as a volunteer secretary, organized and engineered an "International Congress of Medical Laboratory Technologists" in 1954, and it is she who, by various means, has contacted and encouraged technologists from many countries in what we may hope is the beginning of a world-wide sharing of knowledge, techniques, and interests relative to our profession.

Some two and a half weeks later I again met Miss Pletscher in London, in the office of the General Secretary of the British Institute of Medical Laboratory Technology, Mr. Richard J. Lavington. This was the introduction to a week-long opportunity to share experiences, interests, ideas and ideals—with representatives of our profession from France, Germany, Holland, Denmark, Spain, Switzerland, Sweden, and Great Britain—these last coming from as far afield as England, Scotland, Ireland, and even Nigeria. Our gracious hosts, the members of the BIMLT, gave the "guests from other countries" (I've never liked the word "foreigner"—it doesn't sound friendly) ample opportunity to share their Seventh Triennial Conference activities.

I was especially interested in comparing notes on their convention—from the viewpoint of a spectator—though the terminology may be different, the basic underlying principles are ever so similar.

The scene of the Conference was the University of Nottingham—the guests were housed in dormitories. The exhibits were in the Science Building—the scientific papers were given in the main auditorium—coffee was served during morning breaks in the program—and tea in the afternoon. And we literally walked MILES upon MILES between buildings. And our technical exhibitors "eye us askance" if the exhibits are not housed "next door" to the auditorium! (And I had more trouble with translating English English into American English than I did anything else since after all, I KNEW I didn't know any other language, but ENGLISH!)...

Our hosts had prepared an outstanding exhibition in a downtown Nottingham Technical College—demonstrations of various laboratory procedures were beamed to interest the public. This was a feature we could well introduce into our own recruitment and public relations activities. Conference-wise, the meeting was thoroughly enjoyable—the papers were of high calibre, some more practical than others—but that is always the case —The "commercial" and scientific exhibits were comparable to those we find at our own conventions, although the general layout was quite different. Instead of being in booths, each exhibit was laid out on the science laboratory tables. Attendance at sessions was good—so altogether it seems that medical technologists from other countries as well as our own are avid for new information and for the sharing of their knowledge and contacts.

The conference and local committees had an entertainment program that showed an immense volume of planning and effort—how the Transportation Committee functioned! There were "tours" without end—the greatest problem was to get oneself organized so as not to miss anything.

With at least 95% of their members being men, the BIMLT is faced with providing "entertainment for the ladies," the wives of the technologists—a problem very few other technologist associations have. (It made us appear rather remiss as we usually leave the husbands or wives attending our meetings to fend for themselves). The Conference visitors were received at the Council House in Nottingham by the Lord Mayor and the Sheriff of Nottingham and their ladies as a welcome to the city. Two dances, one for getting acquainted, the other as the "grand finale" following the banquet, the many interesting tours, scientific as well as industrial —the Sunday afternoon drive to Derbyshire for sightseeing—made the entertainment program complete.

There were opportunities to discuss medical technology, with men and women interested in its progress, salaries, recruitment—in a general meeting with a large number of the British members which reminded me of our Public Relations or Recruitment conferences, in the committee meeting with just the appointed delegates present, or in informal sessions with groups of Swiss, or German, or French, or Dutch, or perhaps people from any two or three of the countries represented—even each mealtime provided company for such discussions evolving from our common interests.

The fact that we bridged our language differences so easily—because of the oneness of our professional interests—made this an unforgettable occasion for me—one upon which I feel medical technology can build. These associations of medical technologists, "without interference with the autonomy of the constituent societies," can "establish and maintain communication among the societies of medical laboratory technologists in the countries of the world," and can "promote cooperation among them."[1] We are looking forward to future meetings with our friends from medical laboratory technologists' societies from these and other countries. We hope that the American Society of Medical Technologists will participate in carrying out the objectives as set forth in the committee meeting:

"To form a closer relationship between the constituent bodies,
To encourage an exchange of periodicals,
To enlist the support of societies in countries not already represented,"
thereby, to crystallize service and progress through cooperation.

R. M.

[1] From the Minutes of a Meeting of Representatives of the Various Organizations present at the 1955 Nottingham Conference of the Institute of Medical Laboratory Technology.

THE PARABLE OF ANTEEN AND THE WISE MAN

AMOS CAHAN, M.D.

Once upon an afternoon dreary and it happened not so long ago, there worked in the laboratory of a hospital to help the sick, a sweet young lady named Anteen. Her hospital was not very large and Anteen did full many a routine test until she felt that the years of her life were passing her by and that she was not getting anywhere.

And Anteen sighed and thought, "Oh, I do wish that my chiefs were more inspiring and interested in me and my work. Nobody pays any attention to me. I'd like to catch me a man, a dark handsome man, who'd love me and take care of me because I'm tired of taking care of everyone."

With that, her wish raced up into the sky and brushed upon a star and

the star burned sanguine and the clock on the wall tolled 4:44 and in walked Tom.

"Two more pints of blood for Mrs. Aronowitz, please, and it's an emergency." His eyes sparkled with laughter.

Anteen thought, I'd like to find out what makes him laugh all the time. She said, "It sure is an emergency—it's five o'clock. And tonight I'm going out to dinner. Oh dear, what do they do all day?"

She felt Tom's eyes watching her as she set up the cross matches. Well, that early date—only too true it was for another supper by herself. She looked at Tom from the corners of her eyes and they burned brighter and she tossed her straight hair back with her fingers. Then she read the cross matches.

First one: compatible. Second one: incompatible.

Anteen quickly and skillfully checked all the blood typings. They were correct. She rechecked the cross match. It was still incompatible. She cross matched another pint. This pint was compatible and she handed the two good pints to Tom whose eyes sparkled with brightest stardust as he watched her. Anteen put all four tubes (funny about that incompatible one) back into the refrigerator and it was just after five as she stepped onto the sidewalk in front of the hospital.

"Anteen listen," her star called and the voice echoed and reechoed from all the hollow caverns in the sky. "Anteen listen—that second tube—that's your discovery—it's the answer to your prayer—don't ignore that incompatible cross match. You are the first to see this new and undiscovered blood factor. Will you be congratulated, Anteen?"

The streets around her rushed and roared with the noise of the five o'clock traffic and Anteen could not hear anything else.

"Anteen," the crossed star pleaded, "why not report the incompatibility to your Chief and suggest sending the specimens to the Wise Man who sits in the Ivory Tower? He will square the riddle that circles the enigma. Anteen, I've sent you your chance. Are you prepared? Don't, please don't let your discovery get away from you."

Now as you shall see, Anteen was not dull by any manner of means for the thought came to her suddenly as she walked through the park. The next morning she sent the tubes to the Wise Man who solved the riddle and they became famous and lived happily ever after. That is to say, she and Tom, not the Wise Man.

My goodness, he is still sitting happily in his Ivory Tower and is now waiting for **your** incompatible crossmatches.

The End.

FROM OUR READERS:

Reginald J. Bromfield, FIMLT, Winchester, England—

"With reference to Cleola M. Wurth's note on the use of pooled serum for protein standards (AMER. JOURNAL OF MEDICAL TECHNOLOGY 1955, 21, 309). For some time I have used a sample of 'out-of-date' dried plasma as a permanent protein standard. This is reconstituted by preparing a seven per cent solution in distilled water, any slight opalescence being ignored. The total protein is estimated by Kjeldahl, and if kept in a well stoppered bottle, or better still, in vacuo, the protein content of the dried plasma will remain constant for years."

ABSTRACTS

p-TOLUENESULFONIC ACID, CHOLESTEROL DETERMINATION, AND EXPLOSIONS.

Betty Jo Jones and Ferrin B. Moreland. (Baylor University College of Medicine, Houston, Texas) Clin. Chem. 4, 345 (1955).

This usually smooth running determination developed explosive properties on a recent occasion. Consecutive use of two freshly prepared solutions of p-toluenesulfonic acid resulted in the contents being blown out of the tubes. Complete examination was not made of the stock bottle of solid but notice was taken of the moist condition of the solid, particularly inside the screwcap.

When a new, unproved solution is prepared it is advised to set the rack of tubes under a shelf or to cover them with a board immediately after the components are added.

SIMULTANEOUS DETERMINATION OF pH, CO_2 CONTENT, AND CELL VOLUME IN 0.1 ML. ALIQUOTS OF CUTANEOUS BLOOD. MODIFICATION OF THE SHOCK AND HASTINGS TECHNIC.

Richard B. Singer, et al. (University of Pennsylvania) Clin. Chem. 4, 287-316 (1955). A modification which holds as its advantages the collection and handling of blood without use of oil; a modified design of the micropipets so that pH readings can be obtained in a filter photometer with a standard error of measurement of a single reading of ± 0.009 with reproducibility of the photocolorimetric pH measurement slightly better than with the most accurate commercial glass electrodes and of ± 0.22 mM/L for the whole blood CO_2.

Results presented confirmation of the fact that arterial and cutaneous blood are virtually identical in acid-base properties; that blood can be handled in the presence of brief, limited, exposure to air with no detectable change in pH and negligible change in CO_2 content (decrease less than 0.2 mM/L).

THE USE OF AN ACID SOLUBLE PROTEIN FRACTION IN THE FLOCCULATION TEST FOR THE DIAGNOSIS OF TRICHINOSIS.

Elvio H. Sadun and L. Norman. (Communicable Disease Center, Atlanta, Georgia) J. Parasitology 41, 476-82 (1955).

An acid soluble protein fraction antigen and a whole larval antigen were prepared and used with similar results in a flocculation test to determine trichinosis in human, hog and rabbit serums. Advantages of the acid soluble fraction were noted: it could be stored indefinitely, somewhat greater sensitivity and it gave a test easier to read and interpret.

A HISTOCHEMICAL PROCEDURE FOR LOCALIZING AND EVALUATING LEUKOCYTE ALKALINE PHOSPHATASE ACTIVITY IN SMEARS OF BLOOD AND MARROW.

Leonard S. Kaplow. (University of Vermont and State Agricultural College) Blood 10, 1023-9 (1955).

The presence of alkaline phosphatase activity in the cells in blood and bone marrow were shown. Fixation with 10% formalin in methanol at ± 5 degrees C for 30 seconds and staining by the azo dye procedure with propanediol buffer at a pH of 9.6. Comparison studies with cells from normal persons was made by a "scoring technic." Normal adults showed no staining in 43-95% of all neutrophils, in patients with pathologic disorders the staining was more intense.

GLUCOSE-INSULIN TOLERANCE TEST IN RHEUMATOID ARTHRITIS.

Jacques Houli and Helion Povoa Filho. (University of Brazil) J.A.M.A., 159, 561-2 (1955).

Twenty-nine cases of rheumatoid arthritis were investigated as to the possibility of adrenal or pituitary insufficiency. Twelve were examined by the method of Fraser and co-workers and seventeen by that of Engel and Scott. The two methods are given. Results of the study were normal in the two groups of patients.

CONTINUOUS TISSUE CULTURE OF LEUKOCYTES FROM HUMAN LEUKEMIC BLOODS BY APPLICATION OF "GRADIENT" PRINCIPLES.

Edwin E. Osgood and John H. Brooke. (University of Oregon Medical School) Blood 10, 1010-22 (1955).

Two new methods of tissue culture made possible the continued growth from six months to one year of cells isolated from human blood. One strain was from a patient with acute leukemic monocytic leukemia and one strain from chronic leukemic granulocytic leukemia. The two methods are outlined.

Results showed that cells resembling tissue histiocytes, Dorothy Reed cells and Langhan's giant cells which were cultured from blood of acute monocytic leukemia supports the view that the monocytic series and the histiocytic series (reticulo-endothelial cells) are one and the same.

The Citadel

COME TO QUEBEC

June 17 to June 22, 1956

Theme: Service through Cooperation

Of course you are making plans to come to Quebec City in June. You will want to be there because this is ASMT's **first** joint meeting with the Canadian Laboratory Technologists and it is the First North American Conference of Medical Laboratory Technologists.

We wonder how many American technologists have been to Quebec. We know if you have ever been there you will want to go back and if you haven't been there, here are some things you should know about this beautiful city.

Quebec City, the only walled city on this continent, has never really left Europe behind. You will have fun exploring its narrow streets, dining on epicurean food and enjoying the view from Dufferin Terrace in front of the Chateau Frontenac, one of the most famous hotels in the world and our convention headquarters.

From the Chateau Frontenac you will see the quaint Lower Town with its ancient buildings and winding streets. You will want to walk through the narrowest street in the New World called "Sous le Cap" (Underneath the Hill). You will see the churches where the French Mariners worshipped, the robed Capuchin friars, and the beautiful Montmorency Falls that are higher than Niagara.

If you are driving to Quebec you will want to go outside the city itself and explore the many quaint little villages.

We will tell you more about Quebec in the next issue of the Journal. Meanwhile begin now to prepare your pathologist and your laboratory for your absence in June.

Beth Armsey, Publicity Chairman

GENERAL INFORMATION

Accommodations

Internationally-known Château Frontenac, the stopping-off place of many a world figure, is as excellent a hotel as can be found anywhere. Other excellent hotels include: the Clarendon, St. Louis, Hotel Château Laurier and Château Normandie, near the Château in Uppertown, and the Victoria, Montcalm, St. Roch and Château Champlain. Wonderful motels abound at the outskirts of the city.

Amusements

Québec in summer offers many amusements. There is baseball at the Stadium, races at the Exhibition Grounds, wrestling and boxing events at 'La Tour', concerts on Dufferin Terrace, the famous Boardwalk in front of the Château Frontenac, where weekly are also held the popular French-Canadian folk-dances in which you join. At the various small nightclubs, the visitor may see floor shows with a distinct French flavor. There are beaches and lakes for water sports; bridle paths for horseback riders.

Eating Places

Visitors to Québec will find many places to dine for every taste and purse. There are places of unique French-Canadian atmosphere where the famous "soupe aux pois" and the "ragoût de pattes" are almost staples of the 'cuisine'.

Transport

Getting around Québec is easy whether the traveller takes a bus, a taxi, calèche or whether he goes about in his own automobile. Québec is easy of access, with regular service to and from the city by rail, bus, steamer and airplane.

Shopping

Handicraft homespuns, hooked rugs and handwoven blankets are a specialty. English china, imported tweeds, linens and woollens can be purchased often at prices much lower than in the United States.

Temperature

The average temperature during the summer months seldom exceeds 75° F. As a rule, nights are cool and it is suggested that the traveller bring along a medium weight coat.

Postal Information

Canadian stamps must be used. Rates: 5 cents on letters destined outside Québec City; 4 cents on postcards.

Exchange of U. S. Funds

U. S. currency is accepted everywhere in payment for goods and services. One may exchange his U. S. currency (or travelers' cheques) as his journey proceeds or as his needs arise. Since the Canadian dollar may at times be at a premium, it is necessary that a slight exchange and banking charge be made. The official rate of exchange varies daily and the current rate is applicable. Any Bank will exchange U. S. or Canadian currency at the prevailing market rate.

CUSTOMS INFORMATION

CUSTOMS: Canadian and United States Customs officials examine all passengers' baggage when crossing the border, on trains or at terminal points without delay or inconvenience.

ENTERING CANADA: No passports are required, however, U. S. citizens by naturalization should carry their naturalization certificates, and citizens of other countries are required to establish their legal admissibility to the U. S. by producing re-entry certificates.

RETURNING TO THE UNITED STATES: The United States Department of State advises that, "The new United States regulations impose no additional requirements on United States citizens and they may, as heretofore, proceed to Canada and return to the United States without

any specified documentation. They need have only as a matter of convenience the documentary evidence customarily carried in the past." American citizens require no documentation and should have no difficulty, through answers to questions, in satisfying the Examining Immigration Officer. In case they are asked to show papers to establish evidence, the possession of birth or baptismal certificate, voter's certificate, tax bills, letter of identification from a bank manager or responsible municipal official, personal letters, car license, driver's license or similar papers will be helpful.

Resident aliens must have Alien Registration Cards, Form I-151.

PERMIT OF PURCHASES: Visitors who have been in Canada 48 hours, may take back to United States, duty free, merchandise to the value of $200.00; if in Canada 12 days or over an additional $300.00 value, provided no claim has been made for similar exemption within six months prior to return from this trip.

These articles which do not include distilled spirits, wines, malt liquors, or cigars, must have been purchased in Canada for personal use, not for re-sale.

DON'T MISS BUFFET AND TEA

There will be no ordinary cup of tea with sandwiches nor a table of cold cuts. Instead you will see a superb work of art of the cuisine which the Chateau is famous for the world over. Be sure to make your buffet and tea reservations early.

NO U. S. SCIENTIFIC EXHIBITS

There will be no scientific exhibits by the members of A.S.M.T. at the Conference in Quebec because the requirements of both the Canadian and American customs make it prohibitive.

NO CONVENTION SEALS

There are no convention seals for sale this year. However, mailing envelopes from A.S.M.T. and C.S.L.T. executive offices will carry a Pitney-Bowes Stamp Meter mark reading: SEE YOU IN QUEBEC DATES JUNE 17-22. Watch for postmark on envelopes from A.S.M.T. office.

Be sure to save the pamphlets and colored brochures you receive from Canada after March 1. They will contain valuable tourist information which you will want to have while you are in Quebec.

For those attending the A.S.M.T. House of Delegates Meeting, please note it will be held June 22. Mark your hotel reservation card accordingly.

POINTS OF INTEREST IN QUEBEC

1. St. Lawrence River.
2. The Citadel.
3. Dufferin Terrace.
4. Wolfe—Montcalm Monument.
5. Chateau Frontenac.
6. Champlain Monument.
7. Post Office.
8. Place d'Armes.
9. Court House.
10. Anglican Cathedral.
11. Ursuline Convent.
12. St. Louis Gate.
13. Cross of Sacrifice.
14. Drill Hall.
15. Parliament Buildings.
16. Museum.
17. Wolfe's Cove.

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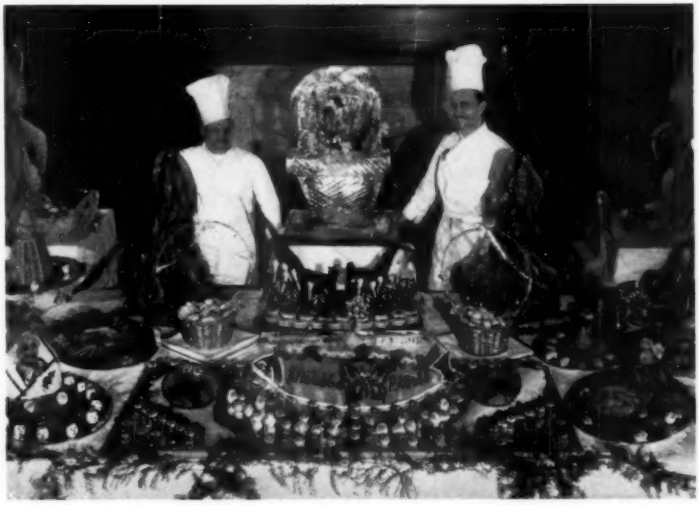
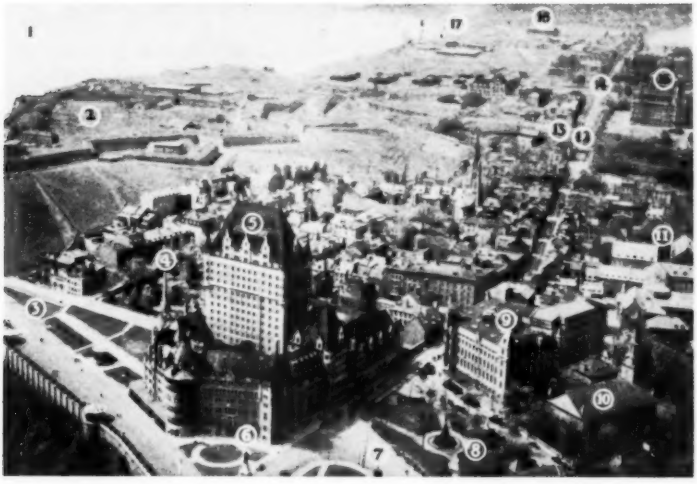


Easter Buffet with Chefs, Chateau Frontenac Hotel, Quebec.



All photographs of Quebec, courtesy of Canadian Pacific Railway Company. Printed in Canada.

FROM THE PROGRAM COMMITTEE

There will be a SYMPOSIUM on **Enteric Diseases** with special emphasis upon techniques for diagnoses in cold climates, with F. KAUFFMANN, M.D., Chief, International Salmonella Center, State Serum Institute, Copenhagen, Denmark, and H. J. FOURNELLE, Ph.D., Chief, Department of Bacteriology, Arctic Health Research Laboratory, USPHS, Anchorage, Alaska, as two members of the panel.

CONFERENCE DEMONSTRATIONS will be on **Public Relations and Recruitment** and **Hospital Accreditation** (Canadian).

There will be ten REFRESHER COURSES:

BIOCHEMISTRY (2 courses)

1. **Biochemistry**—the subject matter of this course cannot be stated in detail at this time. It will be on a practical level to provide assistance to the technologist in the smaller hospital and will include some review of various quantitative and qualitative analyses with emphasis on errors of methods.

2. **Spectrophotometry**—highlighting calibration of instruments, setting up standard curves, sources of error in current methods. Conducted by Elizabeth Frame, Ph.D., Chief Clinical Chemistry Service, Clinical Pathology Department, National Institute of Health, Bethesda, Maryland. Assisted by Jane Egan, B. S., M. T. (ASCP), Clinical Pathology Department, National Institute Health, Bethesda, Maryland.

HEMATOLOGY (2 courses)

1. **A general course** on normal and abnormal cytology. Conducted by Harold Amy, B.A., R.T., Department of Hematology, Hamilton General Hospital, Hamilton, Ontario.

2. **Sternal Marrow**—a laboratory course to be limited to 30 students. Conducted by Jean Marie Delage, M.D., Associate Professor in Hematology, Laval University, Chief of Hematology Department, Saint-Sacrement, Quebec, P. Q.

Please use special application form for this course. (see page 64.)

BACTERIOLOGY (1 course)

A general course on methods of identification. Conducted by Leo Gauvreau, M.D., Associate Professor Bacteriology, Laval University; Chief Bacteriology Department, Saint-Sacrement Hospital, Quebec, P.Q.

SEROLOGY (1 course)

Lectures and demonstrations on:

Test procedures of syphilis serology. Modern trends in syphilis serology. Tests for syphilis using Treponemal antigens. Causes for discrepancies in serological tests for syphilis. Conducted by Genevieve Stout, M.A., M.T. (ASCP), Chief of Field Services Unit Venereal Disease Research Laboratory, USPHS, Chamblee, Georgia.

HISTOLOGY (1 course)

Practical everyday problems, special stains, histochemical methods. Conducted by Evelyn Ballot, M.A., M.T. (ASCP), Supervisor and Instructor, Armed Forces Institute of Pathology, Washington D.C.

BLOOD BANKING (1 course)

Latest advances and trends. Conducted by Mr. Glenn Hill, Associate Director, Division of Serological Development, Ortho Research Foundation Laboratories, Raritan, New Jersey; Mr. Robert T. McGhee, Associate Serologist, Division of Serological Development, Ortho Research Foundation Laboratories, Raritan, New Jersey.

THERE WILL ALSO BE TWO REFRESHER COURSES CONDUCTED IN FRENCH on general fundamentals of laboratory techniques.

ADVANCE REGISTRATION for the FIRST NORTH AMERICAN CONFERENCE of MEDICAL LABORATORY TECHNOLOGISTS, ANNUAL CONVENTIONS of the AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS and the CANADIAN SOCIETY OF LABORATORY TECHNOLOGISTS

June 17-22, 1956—Chateau Frontenac, Quebec, P.Q., Canada

Please fill in and mail with your check or money order to Miss Mary Nagai, M.T. (ASCP), 25 Hermann Professional Building, Houston 25, Texas. If you do not register for a Refresher course, your advance registration application and fees will be accepted if mailed on or before May 20, 1956.

NAME_____ DATE_____
Please Print

BUSINESS ADDRESS_____

city zone state or province country

REGISTRATION FEE $5.00

No. tickets

____	Buffet & Reception	$9.00
____	Luncheon	$3.00
____	Sunday Tea	$1.75
____	Thursday Tea	$1.75
____	Instrumentation Demontration	$ .50
1	Refresher Course	$5.00
	Total	

Do not write in this space

Make your check payable to the First North American Conference of Medical Laboratory Technologists

Please note: Member { ASMT ☐ CSLT ☐ Student { ASMT ☐ CSLT ☐ Visitor ☐

Student members of ASMT will have the Registration fee refunded unless they sign for a refresher course.
ALL REFRESHER course registrations must be mailed by April 15, 1956.
I wish to enroll for a refresher course.
REFRESHER COURSES—Please Note 1st, 2nd, and 3rd choice: (see full description on page 62).

- ☐ Biochemistry (general)
- ☐ Biochemistry (spectrophotometry)
- ☐ Hematology (general)
- ☐ Hematology (sternal marrow)*
- ☐ Bacteriology
- ☐ Histology
- ☐ Blood Banking
- ☐ Serology
- ☐ General Procedures { Two courses conducted in French

*Limited to 30. Fill in form on page 64.
All advance registration applications must be accompanied by the $5.00 registration fee. Unless receipt is requested, your canceled check will serve as receipt.
Make HOTEL RESERVATIONS DIRECT TO THE HOTEL CHATEAU FRONTENAC —QUEBEC CITY, QUEBEC, CANADA—headquarters. Other hotels in the vicinity are:

Clarendon Hotel, 57 Ste. Anne Street
St. Louis Hotel, 33 St. Louis Street
Chateau Laurier Hotel, 107 Grande-Allee
Chateau Normandie Hotel, 12½ Ste. Anne Street
Victoria Hotel, 56 St. Jean Street

Motels approximately 3 to 3½ miles from the Chateau are:

Motel des Laurentides, 350 Blvd. Ste. Anne
Motel du Pont, Blvd. Wilfred Laurier
Motel Bouchard, 333 Blvd. Ste. Anne
Motel l'Abitation, 2828 Blvd. Wilfred Laurier

BONE MARROW REFRESHER COURSE

Name _____
please print

Address _____
please print city state

Laboratory Address: _____

Department _____

Duties _____

Train students: Yes: _____ No: _____

Approximate number students trained per year _____

Time in Hematology _____
months years

Academic standing:

Senior Matriculation _____
(Canadian)

Student in Approved School _____
(American)

University _____
no. of years

_____
degree

Member of the CSLT ☐
(please check)

Member of the ASMT ☐

I hold a specialist certificate _____

(If so, in what field) _____

I am interested in this course because _____

Further Comments: _____

Signed _____

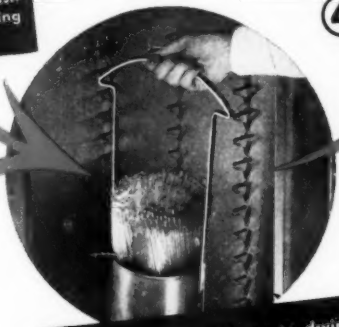
1 Just load dirty pipettes in the basket as they become soiled. Basket rests in cleansing solution.

have lots of clean pipettes always on hand

without drudgery

2 When basket is full, transfer to washer. Three minutes will flush residues away completely, leaving pipettes sparkling clean.

4 Take out basket and use as dispenser for clean pipettes.



3 Remove basket and put in drying oven.

As you can see above, it's no problem at all to have plenty of clean pipettes always available — when you entrust the job to the Technicon Automatic Pipette Washer. Twice every minute the washer swirls seven surging gallons of water through the pipettes inside, flushing away clinging residues. In three minutes (six syphon-cycles) it delivers a full basket-load of immaculate pipettes (holds up to 200 pipettes 1 ml and up to 18" long). The special alloy stainless steel basket is proof against corrosive reagents, even potassium dichromate and sulfuric acid. It carries pipettes as a unit-load through cleansing, washing, drying. Requiring no handling of separate pipettes, it practically eliminates breakage and chipping. Bulletin #6150 gives particulars — let us send it to you.

Pyrex Jar | Pipette Rack | Pipette Washer

technicon

automatic pipette washer

THE TECHNICON COMPANY, Chauncey, New York

Reproducible prothrombin times

with

Solu-Plastin®

(Thromboplastin Solution-Schieffelin)

. . . ACCURATE
. . . DEPENDABLE
. . . CONSISTENT

prothrombin activity determinations

"In our hands this [Solu-Plastin] has been found to be quite stable and yields relative uniform curves from lot to lot."

Pascale, L. R., and Olwin, J. H.: Circulation 9:230 (Feb.) 1954.

EVERY rigidly standardized lot (checked against both normal and dicumarolized human plasma) is ready for use . . . needs only to be mixed with calcium chloride solution as required . . . there is no waste. To assure sensitive, reproducible end points, Solu-Plastin may be used in any of the standard techniques . . . Quick one stage; or two stage; modified Owren.

Solu-Plastin retains full activity indefinitely at 4° C . . . for a minimum of 2 weeks at room temperature . . . somewhat shorter time at higher temperatures.

SUPPLIED: Bottles 10 cc (100 determinations) with like amount of 0.0125 M calcium chloride solution.

Easy-to-follow wall Directions Cards, useful Prothrombin Determination Records and full literature will be supplied on request. Write your name and address on the margin of this advertisement . . . return to:

Write also for the latest progress on Schieffelin's C·R·P·A® (C-reactive Protein Antiserum – Schieffelin) an aid in evaluation of anti-inflammatory therapy.

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